Understanding biological pathways involved in the onset of depression in adolescence

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UNDERSTANDING BIOLOGICAL PATHWAYS INVOLVED IN THE ONSET OF DEPRESSION IN ADOLESCENCE

by

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A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy at King's College London

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Acknowledgments

There are many people I would like to thank for being by my side during this long and amazing journey.

Firstly, I would like to thank my supervisors Dr Valeria Mondelli and Dr Annamaria Cattaneo. Thanks, Valeria, for giving me the amazing opportunity to join the PIXIE Lab, for being so supportive during this entire journey, and for always reminding me to believe in myself. Thanks, Anna, for these years working together, for teaching me to never give up and how to deal with problems, because I learned that there is always a solution even in the worst scenario. Thank both for being a truly inspiring scientists and women, I learned a lot from both of you.

Thanks to the IDEA team for being such an amazing international family. I felt welcome from the first time I joined the team, and I could not be luckier to do my PhD in this amazing team. It was truly inspiring to see how research is done in different countries worldwide and you all help me to develop a truly open-minded approach to research. Thanks to all the IDEA project participants for joining the project.

Thanks to my two labs, the Biopsy and PIXIE lab. Thanks to all my colleagues of the Biopsy Lab in Brescia, for being such an amazing group and for all the time spent together inside and outside the lab. A special thanks to Monica, thanks for being my lab partner in crime and a sincere friend.

Thanks also to my colleagues of the PIXIE lab in London, you welcomed me as part of the group the very first day and you let me feel at “home” far from my home. Thanks

to Zuzanna for her precious support in the IDEA project and for our pink hair. And a special thanks to Luca, a friend more than a colleague, thanks for our time in the lab and see you on the lab's rooftop soon.

Thanks to my lifetime friends Danika, Mara, Valeria, and Roberta. Thanks for celebrating every achievement and for always let me feel loved. A special mention to Danika, thanks for being "my person" no matter what.

Thanks to my family.

Thanks Mum and Dad for being so supportive, for allowing me to follow my dreams and for always telling me how much you were proud of me even when I felt like I did not deserve it. Your support was precious, I own you a lot.

Thanks to my little sister Alessandra for always believing in me, and for helping me see things clearly when everything was a mess. Thanks for being the shoulder to cry on. Once you said to me that I was your role model, but I can truly say that you are mine.

The biggest thanks to my future husband, Dr Andrea Ghisleni (you deserve this). Thanks for teaching me how to pipette for the very first time in my life, thanks for being such an inspiration and for being the most brilliant and passionate scientist that I know. Thanks for your support during these years, for never letting me down, for believing in me when I struggled to do it myself. I would have never been at this end if it wasn't for you.

Finally, thanks to myself: it was hard, I struggled, but I did it. *Per aspera ad astra*.

Abstract

Major Depressive Disorder (MDD) represents the second leading cause of disability worldwide with an onset that typically occurs in adolescence. The burden of depression is particularly heavy during adolescence, since 10-20% of teen-agers experience at least one depressive episode by the end of adolescence and teens suffering from MDD are more likely to develop drugs abuse and suicide ideations. Noteworthy, the burden of depression is even heavier in low- and middle-income countries (LMICs), that comprise 90% of the global adolescent population. Given the fact that the burden of depression in LMICs is worsened by cultural and social barriers, and scarcer healthcare resources are available, there is an urgent need for improving prevention strategies in LMICs. This need was the focus of the Identifying Depression Early in Adolescence (IDEA) project, a multi-disciplinary global mental health consortium with the main aim to help the identification of adolescents at high or low risk of developing depression, particularly in LMICs. This doctoral study was part of the IDEA project, and thus the main aim was to identify the biological pathways mapping both the presence of depression and the risk of developing depression in high-school adolescents from Brazil. Moreover, given that the incidence of depression is higher in females compared with males, the role of biological sex in differently modulating such biological mechanisms was also investigated.

In order to estimate individual-level probability of developing MDD among Brazilian adolescents, the IDEA consortium developed a composite risk score (IDEA-RS), which is based on sociodemographic variables easily assessed directly from the adolescents.

By using this IDEA-RS, a risk-stratified cohort of 150 adolescents aged 14-16 years old were recruited in the city of Porto Alegre (Brazil). This cohort comprised 50 adolescents classified at high risk (HR) of developing depression and with a current diagnosis of MDD, 50 adolescents at HR but without MDD and 50 adolescents at low risk (LR) and without MDD. Two genome-wide gene expression approaches were used for investigating the biological mechanisms differently modulated according to the presence or risk of MDD, specifically microarrays Affymetrix and RNA Sequencing techniques were performed on blood samples.

Biological pathways linked to inflammation and immune system activation resulted to be up-regulated in adolescents with depression compared with adolescents without depression; a greater up-regulation of such inflammatory pathways was particularly observed in the MDD group when compared with the HR group. Moreover, the up-regulation of inflammation and immune system activation in adolescents with depression was more pronounced in females compared with males. On the other hand, a homogeneous panel of biological pathways able to distinguish between HR and LR adolescents without depression was not identified.

In conclusion, the up-regulation of biological pathways associated with inflammation and immune system was identified as main signature mapping the presence of depression in an adolescent cohort of 14-16 years-old in Brazil. These findings might represent an important step towards untangling the complex pathways involved in the pathophysiology of adolescent depression.

Publications

Publications related to this thesis

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Valentina **Zonca**, Zuzanna Zajkowska, Pedro H. Manfro, Laila Souza, Anna Viduani, Annabel Walsh, Sforzini Luca, Johnna Swartz, Rakesh Karmacharya, Helen L. Fisher, Brandon A. Kohrt, Christian Kieling, Annamaria Cattaneo, Valeria Mondelli. Transcriptomic pathways associated with increased risk and presence of adolescent depression. BAP Summer Meeting 2022. 24th-27th July, London. Poster Presentation.

Valentina **Zonca**, Zuzanna Zajkowska, Pedro H. Manfro, Laila Souza, Anna Viduani, Annabel Walsh, Johnna Swartz, Rakesh Karmacharya, Helen L. Fisher, Brandon A. Kohrt, Christian Kieling, Annamaria Cattaneo, Valeria Mondelli. Understanding the biological pathways underpinning adolescent depression in LMIC: the IDEA Project. MQ Mental Health Science Summit. 12th-13th May 2021. Virtual. Poster Presentation

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Abbreviations

Abbreviation	Meaning
µL	Microliter
5-HT	Serotonin
5-HTTLPR	Serotonin-Linked Polymorphic Region
A260	Absorbance At 260 Nm
A280	Absorbance At 280 Nm
ABEP	Associação Brasileira de Empresa de Pesquisa - Brazilian Economic Classification Criteria
ANOVA	Analysis Of Variance
APA	American Psychiatric Association
ARI	Affective Reactivity Index
ARS	Adolescent Resilience Scale
ATM	ATM Serine/Threonine Kinase
AUC	Area Under the Curve
B. subtilis	Bacillus Subtilis
BCL	Base Call
BDNF	Brain Derived Neurotrophic Factor
BH4	Tetrahydrobiopterin
bp	Base Pair
C3	Complement Component 3
C4BPA	Complement Component 4 Binding Protein Alpha
CAMs	Cell Adhesion Molecules
CAR	Cortisol-Awakening Response
CBT	Cognitive-Behavioural Therapy
CDK5	Cyclin Dependent Kinase 5
cDNA	Complementary DNA
CDRS-R	Children Depression Rating Scale
CFH	Complement Factor H
CGAS	Children's Global Assessment Scale
CGI	Clinical Global Impression
CHARGE	Cohorts for Heart and Aging Research in Genomic Epidemiology
COCH	Cochlin
CREB	C-AMP Response Element-binding
CRH	Corticotropin-Releasing Hormone
CRP	C-Reactive Protein
CSF	Cerebrospinal Fluid
CSH2	Chorionic Somatomammotropin Hormone 2
CTQ	Childhood Trauma Questionnaire
CYP26B1	Cytochrome P450 Family 26 Subfamily B Member 1

DA	Dopamine
DEFA1	Defensin Alpha 1
DEFA1b	Defensin Alpha 1 Beta
DEGs	Differently Expressed Genes
DET1	Det1 Partner Of Cop1 E3 Ubiquitin Ligase
DNA	Desoxyribonucleic Acid
DSM	Diagnostic And Statistical Manual Of Mental Disorders
dTTP	Deoxythymidine Triphosphate
dUTP	2´-Deoxyuridine, 5´-Triphosphate
E-RISK	Environmental Risk
E. coli	Escherichia Coli
FC	Fold-Change
FDA	Food And Drug Administration
FDR	False Discovery Rate
FKBP4	FK506 Binding Protein 4
FKBP5	FK506 Binding Protein 5
GABA	Gamma-Aminobutyric Acid
GAPDH	Glyceraldehyde-3-Phosphate Dehydrogenase
GC	Guanine-Cytosine
GR	Glucocorticoid Receptor
GRIA2	Glutamate Ionotropic Receptor AMPA Type Subunit 2
GRIK1	Glutamate Ionotropic Receptor Kainate Type Subunit 1
GRIK4	Glutamate Ionotropic Receptor Kainate Type Subunit 4
GRIN2A	Glutamate Ionotropic Receptor NMDA Type Subunit 2A
GTPase	Guanosine Triphosphate Binding Protein
GWAS	Genome-Wide Association Study
HCPA	Hospital De Clínicas De Porto Alegre
HICs	High-Income Countries
HIV	Human immunodeficiency virus
HPA	Hypothalamic-Pituitary-Adrenal
HR	High-Risk
Hsp90	Heat-Shock Protein 90
HTR2A	5-Hydroxytryptamine Receptor 2A
IDEA	Identifying Depression Early In Adolescence
IDEA-RiSCo	IDEA-Risk Stratified Cohort
IDEA-RS	IDEA Risk Score
IDO	Indoleamine 2,3-Dioxygenase
IFN	Interferon
IL	Interleukin
IPA	Ingenuity Pathways Analysis Software

IQ	Intelligence Quotient
IRF	Interferon Regulatory Factor
K-SADS-PL	Kiddie – Schedule For Affective Disorders And Schizophrenia For School-Age Children – Present And Lifetime Version
KYN	Kynurenine
KYNA	Kyn-Kynurenic Acid
LILRA5	Leukocyte Immunoglobulin Like Receptor A5
LMICs	Low And Middle-Income Countries
LPS	Lipopolysaccharide
LR	Low-Risk
MAOIs	Mono-Amine Oxidase Inhibitors
MAPK	Mitogen-Activated Protein Kinase
MBCT	Mindfulness-Based Cognitive Therapy
MBSR	Mindfulness-Based Stress Reduction
MDD	Major Depressive Disorder
MDQ	Mood Disorder Questionnaire
MFQ	Mood And Feelings Questionnaire
mhGAP	Mental Health Gap Action Programme
miRNA	microRNA
mL	Milliliter
MRI	Magnetic Resonance Imaging
mRNA	Messenger RNA
MRS	Magnetic Resonance Spectroscopy
N	Normality
NA	Noradrenaline
NET	Norepinephrine Transporter
NF-KB	Nuclear Factor Kappa-Light-Chain-Enhancer Of Activated B Cells
ng	Nanogram
ng/µl	Nanogram / Microliter
NIHC	National Institute Of Mental Health
nm	Nanometer
nM	Nanomolar
NMDA	N-Methyl-D-Aspartate
NRCAM	Neuronal Cell Adhesion Molecule
NPY	Neuropeptide Y
NPY2R	Neuropeptide Y Receptor Y2
P53	P53 Tumor Protein
PAK	P21-Activated Kinases
PBI	Parental Bonding Instrument
PBMCs	Peripheral Blood Mononuclear Cells
PCA	Principal Components Analysis
PCR	Polymerase Chain Reaction
PGC	Psychiatric Genomics Consortium

PHQ-A	Patient Health Questionnaire For Adolescents
PIF	Parent Information Form
PLS3	Plastin 3
pM	picoMolar
PTEN	Phosphatase and tensin homolog
PUFAs	Polyunsaturated Fatty Acids
Q1	First Questionnaire Round
Q2	Second Questionnaire Round
QA/QC	Quality Assurance / Quality Control
QUIN	Quinolinic Acid
RAB5	Ras-Related Protein
RFQY	Reflective Functioning Questionnaire For Youths
RIN	RNA Integrity Number
RMA	Robust Multi-Strip Average
RNA	Ribonucleic Acid
RNA-Seq	RNA Sequencing
SCAS	Spence Children's Anxiety Scale
SHAPS	Snaith-Hamilton Pleasure Scale
SLC6A2	Solute Carrier Family 6 Member 2
SLC6A3	Solute Carrier Family 6 Member 3
SLC6A4	Solute Carrier Family 6 Member 4
SNPs	Single Nucleotide Polymorphisms
SNRIs	Serotonin Noradrenaline Reuptake Inhibitors
SMOX	Spermine Oxidase
SOP	Standard Operating Procedure
SOX	Sry-Type HMG Box
SOX5	Sry-Box Transcription Factor 5
SRGAP1	Slit-Robo Rho Gtpase Activating Protein 1
ss-cDNA	Singe Strand Complementary DNA
SSRIs	Selective Serotonin Reuptake Inhibitors
STAR*D	Sequenced Treatment Alternatives to Relieve Depression
STAT3	Signal Transducer And Activator Of Transcription 3
STRBP	Spermatid Perinuclear Rna Binding Protein
TACSTD2	Tumor Associated Calcium Signal Transducer 2
TADS	Treatment For Adolescents Depression Study
TBC1D3	Tbc1 Domain Family Member 3
TBC1D3H	Tbc1 Domain Family Member 3h
TCA	Tricyclic Antidepressant
TdT	Terminal Deoxynycleotidyl Transferase
TNF	Tumor-Necrosis Factor
TRD	Treatment-Resistant Depression
TREM1	Triggering Receptor Expressed On Myeloid Cells 1
TRP	Tryptophan
UDG	Uracil-DNA Glycosylase

VEGF	Vascular Endothelial Growth Factor
WASI	Wechsler Abbreviated Scale Of Intelligence
WEIRD	Westernized, Educated, Industrialized, Rich And Democratic
WHO	World Health Organization
YSI-A	The Youth Strength Inventory – Adolescent Version

1. Introduction

1.1 Major Depressive Disorder

Major depressive disorder (MDD) is a psychiatric condition characterized by discrete episodes of at least 2 weeks duration involving alterations in affect, cognition, neurovegetative functions and inter-episode remissions (American Psychiatric Association, 2013). According to the DSM-5 criteria for Major Depressive Disorder, the diagnosis of MDD require at least 5 of the following symptoms:

- sad mood
- loss of interest or pleasure in all activities
- weight loss or gain
- insomnia or hypersomnia
- psychomotor agitation or retardation
- fatigue or loss of energy
- feelings of worthlessness or excessive and inappropriate guilt
- decreased ability to think or concentrate
- recurrent thoughts of death or suicide (American Psychiatric Association, 2013).

These symptoms must be present during the same two-weeks period and specifically, depressed mood and/or loss of interest and pleasure must be present (DSM-5).

MDD is a global burden and represents the second leading cause of disability worldwide. According to World Health Organization (WHO), 264 millions of people suffer from depression, which represents the first cause of suicide among all the age groups. The burden of depression is particularly heavy in adolescence: the WHO has

estimated that about 10-20% of teen-agers experience at least one depressive episode by the end of adolescence and generally, mental health conditions in adolescence account for 16% of the global burden of disease and injury in people aged 10-19 years old. Adolescents suffering from depression are more likely to develop drugs abuse and suicide attempts (National survey on drug use and health, 2016) and depression has been considered the first cause of suicide among youth (Adolescent Mental Health, WHO, 2018). As it happens in adulthood, depression is more prevalent in girls (around 20%) than in boys (around 6%).

Importantly, the incidence of depression rapidly grew due to the very recent COVID-19 pandemic in all age groups. For example, children and adolescents faced a significant increase in the risk of depression due to school closures, social isolation and quarantine following COVID-19 pandemic (Loades et al., 2020; Nearchou, Flinn, Niland, Subramaniam, & Hennessy, 2020). For example, Pierce and colleagues observed a general increase in mental distress in individuals aged 16 years and older in the UK in 2020 compared with 2019 (Pierce et al., 2020). In an Italian setting of students aged 18-30 years old, Meda and colleagues reported on average worse depressive symptoms during lockdown compared with six months before (Meda et al., 2021). COVID-19 lockdown also negatively impacted children mental health: anxiety, depression and stress were observed to be increased in children after the school closure due to the pandemic (Tang, Xiang, Cheung, & Xiang, 2021) and an increase in children's depression symptoms was observed compared with pre-lockdown period in the UK (Bignardi et al., 2020).

1.1.1 Pathophysiology of Depression

Depression is a complex and heterogenous disorder, as demonstrated by the large variety of symptoms and subtypes of the disorder. Moreover, the complexity of MDD is also suggested by the fact that about one third of patients does not respond to first line antidepressant drugs. The reasons underpinning this complexity might be due to the lack of a unique mechanism underlying the disorder, as several hypotheses were proposed and investigated during the past decades, from the dysregulation of the monoaminergic system and glutamatergic systems to the increased inflammation and immune activation, HPA axis dysregulation, reduced neurogenesis and neuroplasticity, and lastly the involvement of gut-brain axis (Zonca, 2022a). All these mechanisms, that will be briefly discussed in the following sections, were shown to be involved and deregulated in the pathophysiology of depression, and hence it is plausible to believe that they might play a common and additive role in the etiopathogenesis of depression (Zonca, 2022a).

1.1.1.1 The monoamine-deficiency hypothesis

The monoamine-deficiency hypothesis – which was firstly hypothesized in 1950 - implied the depletion of serotonin (5-HT), dopamine (DA) and noradrenaline (NA) levels in the brain. The role of the monoamine in depression was supported by several evidence over the decades, as for example 5-HT, NA and DA were shown to be reduced in *post-mortem* brain as well as in peripheral blood of patients with depression compared with healthy individuals (Kambeitz & Howes, 2015; Moret & Briley, 2011). Moreover, genetic polymorphisms associated with monoamine transporters genes were linked to increased risk of developing depression. For example, s/s genotype of

the serotonin-linked polymorphic region (5-HTTLPR) – which is responsible for the re-uptake of 5-HT in the pre-synaptic region – was shown to reduce the re-uptake of 5-HT in the brain and it was studied as a possible genetic vulnerability factor for the onset of depression (Fratelli, Siqueira, Silva, Ferreira, & Silva, 2020). Among the neurotransmitters, also glutamate was studied in relation to depression, as the deregulation of the glutamatergic system was observed to be involved in the onset of depression via a reduction of neuroplasticity through the down-regulation of the brain derived neurotrophic factor (BDNF). Further supporting a role of glutamate in depression, the NMDA receptor's antagonist Ketamine showed a rapid antidepressant effect after low-dose infusion in treatment resistant patients with MDD (Iadarola et al., 2015; Nikkheslat, 2021).

1.1.1.2 Stress

Stress is defined as "the physiological or psychological response to internal or external stressors; it involves changes affecting nearly every system of the body, influencing how people feel and behave" (American Psychological Association Dictionary).

Stress response is regulated by the Hypothalamic-Pituitary-Adrenal (HPA) axis, which activates several molecular cascades to prevent the disruption of the physiological neural and body activity. Stressful events activate the HPA axis which in turn releases the stress hormone cortisol. The association of HPA axis and the related stress hormone cortisol with depression was widely discussed in literature, and the hyperactivity of the HPA axis in patients with depression was defined as one of the most robust findings in psychoneuroendocrinology (Menke, 2019). Several studies investigated peripheral cortisol levels in patients with MDD, showing various

abnormalities in the HPA activity compared with controls. For example, cortisol's circadian rhythm was shown to be disrupted in patients with depression, as high morning cortisol and flat pattern of cortisol secretion during the day were observed in inpatients with MDD (Dienes, Hazel, & Hammen, 2013), as well as high morning and evening cortisol levels were shown in saliva samples from patients with depression (Vreeburg et al., 2009). Similarly, cortisol-awakening response (CAR) was shown to be blunted in depression as well as in healthy controls with high risk of developing depression (Duan et al., 2013; Huber, Issa, Schik, & Wolf, 2006).

As previously mentioned, HPA axis is activated in response to stress and cortisol production is part of the stress-response pathway; once cortisol is secreted, its production is suppressed by cortisol itself through a negative feedback mechanism. A reduced regulation of this negative feedback loops was proposed to be responsible for the chronic activation of the HPA system, and this chronic activation was suggested as one of the main mechanisms underlying stress-induced depression. Further supporting this hypothesis, it is noteworthy that the glucocorticoid receptor (GR) sensitivity was shown to be reduced in patients with depression compared with controls, leading to the hypersecretion of corticotropin-releasing hormone (CRH) and cortisol, due to the lack of negative feedback mechanism (Huber et al., 2006; Mikulska, Juszczyk, Gawronska-Grzywacz, & Herbet, 2021).

Moreover, the deregulation of HPA axis and stress was observed to be responsible for neuroplasticity disruption, which was in turn linked to development of depression. Specifically, sustained exposure to elevated levels of cortisol was shown to induce negative consequences on hippocampal neurons, such as reducing dendritic branching and inhibiting neurogenesis (Kim, Pellman, & Kim, 2015). Decreased

hippocampal volume was described in women who experience sexual trauma during childhood and in women with depression with a history of child abuse (Murphy et al., 2022; Quide et al., 2018; Teicher et al., 2018), suggesting a smaller hippocampus volume in patients with MDD.

1.1.1.3 Immune system

The inflammatory hypothesis of depression, also known as the monocyte-T-lymphocyte or cytokines hypothesis of depression, was proposed following the description of the so-called sickness behavior, which is a syndrome characterized by depressive-like symptoms such as social withdrawal, decreased appetite, anhedonia, lethargy, impaired concentration, depressed mood, irritability, and increase sensitivity to pain and fever (Dantzer & Kelley, 2007). A large body of studies demonstrated increased inflammatory cytokines and receptors, acute-phase proteins, chemokines, and adhesion molecules in peripheral tissue, such as blood and saliva, in patients with depression compared with healthy controls (Dowlati et al., 2010; Felger & Lotrich, 2013; Zonca, 2022a). Moreover, an hyperactivation of the inflammatory pathways in central nervous system was observed in *post-mortem* brain tissue of individuals with depression and victims of suicide (Pandey, Rizavi, Zhang, Bhaumik, & Ren, 2018). The connection between inflammation and depression paved the way for novel drug discovery approaches targeting the inflammatory system, specifically targeting cytokines as new pharmacological target for antidepressant drugs.

The inflammatory hypothesis of depression was shown to be interconnected also with the other hypothesis of the mechanisms underlying depression, and a clear crosstalk between inflammation and 5-HT and stress was deeply investigated and studied over

the years, demonstrating once again that all the hypothesized mechanisms underpinning depression are connected among each other.

The increased levels of cytokines and pro-inflammatory modulators involved in the sickness behavior was shown to modulate the production and the re-uptake of brain neurotransmitters, such as 5HT and glutamate. For example, IL-1β and TNF-α were shown to induce the activation of the p38 mitogen-activated kinase (MAPK) which in turn increases the expression of the re-uptake pumps of 5-HT in the brain, leading to the reduction of 5-HT bioavailability (Zhu et al., 2010). Secondly, cytokines were shown to generate reactive oxygen and nitrogen species which in turn reduce the bioavailability of tetrahydrobiopterin (BH4), a key co-factor in the synthesis of 5-HT (Neurauter et al., 2008).

One of the most studied mechanisms through which inflammation may lead to development of depression is the regulation of the enzyme indoleamine 2,3-dioxygenase (IDO), which metabolizes tryptophan (TRP) into kynurenine (KYN) (Jeon & Kim, 2017) (Figure 1.1). The metabolization of tryptophan by IDO produces kynurenine (KYN) which is in turn metabolized via two different routes: the KYN-kynurenic acid (KYNA) pathway and the KYN-nicotinamide adenine dinucleotide (NAD) pathway, which produces quinolinic acid (QUIN); for details refer to (Jeon & Kim, 2017). QUIN is mainly produced in the microglia, whereas KYNA in astrocytes (Guillemin, Smythe, Takikawa, & Brew, 2005). These two metabolites act on the glutamatergic receptor NMDA but via opposite mechanisms: KYNA is an antagonist whereas QUIN is an agonist of NMDA receptors (Stone, 1993).

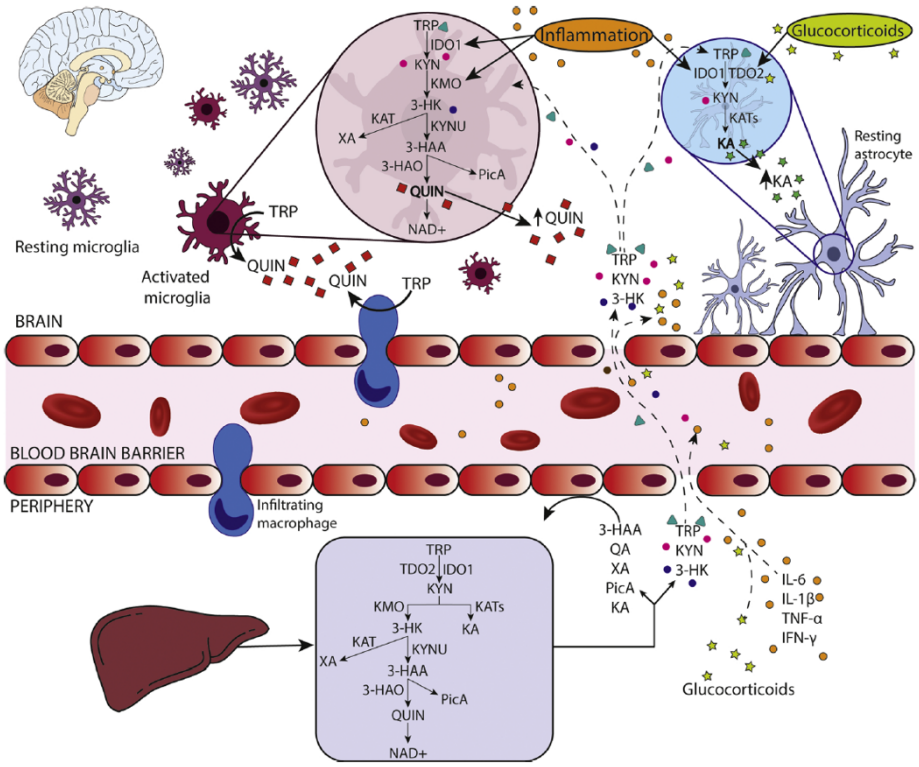
The enzyme IDO was shown to be activated by pro-inflammatory cytokines such as IFN-γ, IFN-α, IL-1, and TNF-α, which metabolized TRP into KYN instead of 5-HT. Since

TRP is the essential amino-acid precursor of 5-HT, and given the fact that IDO reduces its availability by producing the metabolite KYN, the kynurenine pathway was shown to play a key role in the transition from sickness behavior to depression-like behavior in LPS-treated mice (O'Connor et al., 2009). Reduced circulating levels of TRP were observed in cancer patients treated with IL-2 and IFN-α (Capuron et al., 2002) and the severity of depressive-like symptoms in these patients were positively correlated with the magnitude of decrease in TRP concentration during the treatment, suggesting that depression onset and symptomatology might be influenced by TRP levels and, as a consequence, by the levels of the 5-HT. To better understand whether TRP might be useful for treating or preventing depression, the effect of diet supplementation of TRP was investigated, but despite initial positive premises this approach was not successful. On the other hand, acute TRP depletion was shown to produce a decrease in brain 5-HT levels and lower mood (Young, 2013). However, no evidence was found to prove a connection between decreased brain TRP and 5-HT levels in inflammation-associated depression.

Furthermore, TRP was also shown to interact with the glutamatergic system via the kynurenine pathway. It has been proposed that the equilibrium between these metabolites is disrupted by cytokines, such as IFN-γ , TNF-α and IFN-α (Widner, Ledochowski, & Fuchs, 2000), inducing neurotoxicity by promoting microglia activation and down-regulating astrocyte activity (Muller, Myint, & Schwarz, 2009). Specifically, the accumulation of QUIN produces a hyper glutamatergic status which was associated with depression and it was also observed to promote hippocampal atrophy and to impair the physiological negative feedback regulation of the HPA axis, due to the reduction of corticosteroid receptors (Wichers & Maes, 2004). Lastly,

increased levels of glutamate in the brain were shown to be responsible of reduced levels of some neurotrophic factors, such as BDNF (Hardingham, Fukunaga, & Bading, 2002). This imbalance might explain the connection between inflammation and depression, as reduced levels of BDNF were described in patients with depression compared with control individuals (Myint et al., 2007).

Figure 1.1. Schematic representation of the kynurenine pathway in human periphery and brain. Figure from (Brown, Huang, & Newell, 2021).



1.1.1.4 Neuroplasticity and neurotrophins

Changes in neuroplasticity and brain size in patients affected by depression were observed (Caviedes, Lafourcade, Soto, & Wyneken, 2017), and *post-mortem* and *in vivo* studies described a reduction in hippocampal volume, glial cell loss, neuronal atrophy and synaptic loss in patients with depression (Cobb et al., 2013). Similarly, reduced hippocampal volume was observed in suicide attempters with MDD compared with non-suicidal patients with MDD (Colle et al., 2015). Similar findings were observed also in younger populations; for example, bilateral reduced grey matter density in the hippocampus and a reduction in the volume of the left hippocampus was found in girls at high risk of developing depression compared with low-risk girls (Chen, Hamilton, & Gotlib, 2010).

1.2 Gene expression studies and Depression

1.2.1. The interplay between genes and environment

Together with the mechanisms underlying the pathophysiology of depression that were acknowledged in the previous chapter, also genetic variants must be discussed in relation to depression. Indeed, a milestone in the pathophysiology field is the interaction Gene X Environment (G X E), that investigates the interplay between genetic variants and environmental risk factors (such as childhood trauma) in the pathophysiology of MDD. Indeed, twin studies suggested that 35-45% of variance in risk for MDD depends on genetic factors, and several studies investigated the genetics of mood disorder by using genome-wide analysis (Sullivan, Neale, & Kendler, 2000). However, difficulties in identifying and replicating the studies on genetic variants associated with MDD were widely observed over the years, and these difficulties were explained by mainly pinpointing the role of the environment and by the G X E interaction. This interaction was observed in the early 2000s by Caspi and colleagues, who argued that previous inconsistencies of polymorphisms and candidate gene findings were due to effect of the environment exposure (Caspi et al., 2002). Specifically, they showed as an example that the impact of the 5-HTTLPR polymorphisms on depression was moderated by exposure to stress early in life, and specifically that the positive association between stressful life events and depression was stronger in individuals carrying the short allele (Caspi et al., 2003).

As previously mentioned, genome-wide association studies showed significant difficulties in identifying vulnerability genes for MDD, as for examples no significant findings were observed in the Psychiatric Genomics Consortium (PGC) study or in the CHARGE meta-analysis of depressive symptoms. The reasons underlying these

difficulties lied in the fact that MDD is influenced by many genetic loci and thus the effect appear smaller (Wray et al., 2018). Hall and colleagues conducted a genome-wide meta-analysis of stratified depression in two large British cohorts, the Generation Scotland, and the UK Biobank, by conducting an unstratified analysis followed by a subsequent analysis stratified based on the recurrence of MDD or biological sex. In this study, the authors identified one genome-wide significant locus only in MDD males but not in relation to females or to the recurrence of MDD (Hall et al., 2018). Recently, a genome-wide association meta-analysis based on more than 135 thousands of cases and 344 thousands of control was able to identify 44 independent and significant loci as associated with clinical feature of MDD (Wray et al., 2018). Nevertheless, very recently Coleman and colleagues performed a meta-analysis on the PCG genome-wide association studies of MDD and bipolar disorder, by also adding data from an additional major depressive disorder cohort from the UK Biobank. This meta-analysis resulted in seventy-three loci reaching the genome-wide significance, including also novel genes that were never identified before. Among those loci, the majority were associated with MDD only, and those that were different between MDD and bipolar disorder were pinpointed by the authors as the results of the epidemiological heterogeneity rather than biological mechanisms (Coleman et al., 2020).

Together with GWAS studies, also transcriptomic and candidate genes approaches were used to identify possible biomarkers and mechanisms underlying depression. A wide plethora of studies were conducted on a candidate-gene basis to identify possible biomarkers. However, almost all large-scale genome wide association studies failed to detect genes at a genome-wide significance level. Similarly, also candidate

gene studies showed a small replicability in terms of genes associated to MDD. For example, in the replication study conducted by Bosker and colleagues in 2011, they were able to replicate only a very small percentage of the previous studies, specifically only 7% of genes and 3-4% of SNPs (Bosker et al., 2011). More recent literature tried to review and replicated the candidate gene studies on MDD, as the one published in 2016 by Luo and colleagues, in which the authors conducted a systematic review of genetic association studies on MDD published between 2007 and 2012. Their analysis resulted in the replication of only 9 SNPs and in the replication of 18 genes (Luo et al., 2016). Similarly, a very recent systematic review of candidate genes for MDD was published by Norkeviciene in 2022, which continued the work of Luo and colleagues, thus exploring data on candidate gene for MDD from 2012 to 2019 (Norkeviciene et al., 2022). However, only a few polymorphisms and genes were studied and identified after the 2012 systematic review of Luo, suggesting a diminished interest in investigating candidate gene as biomarkers for MDD. Moreover, in their study they confirmed 18 genetic polymorphisms, and 23 genes were identified to be related to MDD. Moreover, following a pathways analysis, their analysis showed that most genes were involved in signaling transmission, specifically glutamate neurotransmission, as among those genes they identified the glutamate receptors GRIA2, GRIN2A, GRIK1 and GRIK4. On the other hand, further genes were shown to encode for proteins involved in neurotransmission through the regulation of calcium channel activity, such as the neuropeptide Y (NPY) and neuropeptide Y receptor Y2 (NPY2R), whereas other genes were responsible for monoamine transporter activity (such as SLC6A2, SLC6A3, and SLC6A4). Lastly, further genes were involved in apoptotic mechanisms, indicating

that apoptosis might be considered an important metabolic pathways involved in MDD (Norkeviciene et al., 2022).

In the scenario of identifying the mechanisms underlying the onset of depression, several studies were focused on identifying a panel of biomarkers suitable for a clear identification of depression. Indeed, some studies identified a panel of genes able to distinguish between depressed and non-depressed individuals. A classifier tool for MDD was developed by using a panel of 7 genes, which was shown to discriminate MDD patients from control with a sensitivity of around 77% and specificity of around 72% (Spijker et al., 2010). Similarly, in a stimulated blood-based genome-wide approach, Menke and colleagues built a classifier for MDD by using a set of 19 genes including for example FKBP5. They observed a sensitivity of 80% and a specificity of 87.5% in discriminating MDD patients from control subjects (Menke et al., 2012). Similar approaches were also applied for predicting antidepressant response in MDD patients (Lin & Tsai, 2016). However, similarly to what previously mentioned, the replication of such biomarkers was not successful.

The very recent study of Mariani and colleagues provide a wide overview of both the candidate and genome-wide studies conducted so far, specifically focusing on the top mechanisms believed to play a major role in the onset of depression, such as inflammation, neuroinflammation, neurotransmission and stress-related mechanisms. Overall, this review showed: 1) a positive correlation between an up-regulated expression of pro-inflammatory genes with the presence of MDD; 2) a dysregulation of neurotrophic and growth factors, such as BDNF and VEGF, as linked with cognitive impairment frequently observed in MDD patients; 3) the involvement of several neurotransmitters and their receptors (specifically the glutamatergic

receptors) in the pathogenesis of MDD; 4) altered expression of stress-related genes in MDD patients, even though inconsistent results were observed. Interestingly, the genome-wide gene expression studies reviewed in the work of Mariani and colleagues, confirmed the association between disfunctions in the inflammatory and stress response as well as in neuroplasticity and neurotransmitters pathways with depression (Mariani et al., 2021).

However, discrepancies were clearly observed in the studies conducted so far, and such discrepancies in the G X E interaction were deeply highlighted by Border and colleagues in 2018. Specifically, the authors examined multiple types of association between 18 highly studied candidate genes for MDD, such as BDNF, SLC6A4, and HTR2A. Surprisingly, they showed that none of the most highly studied polymorphisms within these genes were associated with depression. Moreover, they did not find evidence to support the moderation of polymorphism effect by exposure to traumatic events or socioeconomic adversity, in contrast with the G X E theory (Border et al., 2019). Thus, this study clearly showed results in contrast with the published candidate gene literature.

Overall, although recent literature might have doubted the previous hypothesis associated with depression as well as the interaction of genetic factors and the environment, it is impossible not to acknowledge the many evidence showing a role of inflammation, stress, neurogenesis and monoamines in the onset of depression. Indeed, both aspects might be considered by using hypothesis free approaches for investigating possible new signatures or mechanisms associated with depression, such

as using RNA Sequencing or genome-wide gene expression analysis as it was used in this doctoral study.

1.2.2 Epigenetic changes and depression

Not only genetic but also environmental factors play an important role in the risk of developing MDD as well other psychiatric disorders. Indeed, a plethora of studies have shown that the genetic predisposition with the combination of environmental risk factors, such as exposure to early in life stress, can lead to the onset of depression via epigenetic mechanisms. When talking about epigenetic mechanisms, we can refer to the following definition of Waddingon: ‘‘An epigenetic trait is a stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence” (Waddington, 1940). These epigenetic changes can be mediated by several mechanisms, such as histone modifications, DNA methylation, non-coding RNAs (such as miRNAs), and chromatin remodelling. Although this is not the main focus of this doctoral thesis, as epigenetic modification were not investigated, it is still important to acknowledge that there are several evidences showing the contribution of epigenetic changes in the pathogenesis of MDD. For example, several DNA methylation studies in peripheral tissues showed evidences of differences in MDD patients compared with controls; for example, several studies showed an hypermethylation in the loci encoding for the BDNF and SLC6A4 genes, that were deeply shown to be associated with MDD (M. Li et al., 2019). Moreover, differences in MDD patients compared with controls were also observed with regards to histone modifications and non-coding RNAs. Regarding the latter, several studies also in our research lab focused their attention on the modulation of miRNAs as possible

mediators of the onset of MDD, but also as possible future biomarkers for identifying the presence or risk of depression as well as for predicting the response to antidepressant drugs (Lopizzo, Zonca, Cattane, Pariante, & Cattaneo, 2019). For example, a recent review of 23 studies investigating the levels of miRNAs in peripheral tissues of individual with depression identified 178 different miRNA showing a differential expression in MDD patients compared with controls (Yuan, Mischoulon, Fava, & Otto, 2018). Moreover, miRNAs were also shown to play an important role in the pathogenesis of depression by mediating the effect of early in life stress, which was deeply shown to be a well-known risk factors of MDD (Allen & Dwivedi, 2020).

Overall, these findings showing an implication of epigenetic processes in MDD indicate that both genetic and epigenetic signatures play an additive role in the pathophysiology and MDD, also via their interaction with environmental factors, such as stress early in life (Torres-Berrio, Issler, Parise, & Nestler, 2019).

1.2.3 Omics approaches in depression

In terms of experimental approaches for the identification of mechanisms and biomarkers able to identify and predict the risk and/or the presence of depression, genome-wide gene expression is one of the most used and validated approach, with several studies published investigating the mechanisms underlying the psychopathology in both clinical and pre-clinical models (Cattaneo et al., 2019; Cattaneo et al., 2018; Hepgul et al., 2016). A large number of studies used genome-wide association as the investigating method for the identification of biological risk factors for the onset of depression, both early and later in life (Cattaneo et al., 2013; Pajer et al., 2012; Spindola et al., 2017).The use of -omics approaches, such as

microarrays and RNA sequencing techniques, have surely important advantages. Differently from the candidate-gene approaches – which have a specific panel of target genes to be investigated – the genome-wide approaches are hypothesis-free methods that thus are not biased by previous hypothesis of targeted molecules or mechanisms to be researched, but they analyze the entire genome or transcriptome, allowing to identify novel mechanisms or biomarkers associated with the risk or presence of depression. Secondly, the -omics methods, by exploring the whole genome, allow the investigation of thousands of genes or transcripts at the same time, and they subsequently allow to perform further analysis requiring the information of several genes, such as pathways analysis (as I performed in this doctoral thesis).

Most of the studies conducted so far by using genome-wide approaches used *post-mortem* brain tissue and peripheral blood samples (Mariani *et al.*, 2021), and in this doctoral project peripheral blood samples were used. The use of peripheral blood samples have several advantages, such as the possibility to collect large sample sizes, to easily stabilize RNA directly during the blood withdrawal and it also allows to isolate specific cell subtypes such as peripheral blood mononuclear cells (PBMCs) or leukocytes (Mariani, Cattane, Pariante, & Cattaneo, 2021). The association between brain and the peripheral blood was demonstrated by Sullivan and colleagues (Sullivan, Fan, & Perou, 2006). In their study, they investigated the genes shared among whole blood and sixteen different brain tissues, showing that 60% of transcripts were expressed in whole blood and in at least one brain tissue. Moreover, they observed that both blood and brain tissues have a similar expression profile with regards to genes related to MDD, such as genes related neurotransmitter receptors ad transporters, growth factors, hormones and inflammation (Sullivan et al., 2006).

Since many studies in literature explored the genome-wide approaches to investigate mechanisms underlying mental health disorders and given the advantages of such approaches in identifying potential novel signatures of risk or presence of depression, in this doctoral thesis genome-wide approaches were used as these were shown to be suitable techniques to provide important hypothesis-free data in the context of adolescent depression.

1.3 Adolescent depression

The onset of depression usually occurs during pubertal age and teen depression is one of the leading causes of disability among adolescents. The World Health Organization estimated that about 10-20% adolescents experience at least one depressive episode before the end of adolescence, and this could represent a threat for teen-agers as depressive episodes represent the first cause of suicide among youth (Adolescent Mental Health, WHO, 2018). A report from the National Institute of Mental Health (NIHC) estimated that in U.S. 3.1 million adolescents, aged between 12 and 17 years old, experienced at least one depression episode and this rate significantly increased in 2016 compared with the period between 2004 and 2014. The teen-agers suffering with depression represent 12.8% of their total peers. As previously mentioned, similarly to adulthood, depression is more prevalent in girls (19.4%) than in boys (6.4%) (National survey on drug use and health,2016). Furthermore, adolescents suffering with depression are more likely vulnerable to develop drug abuse and suicide attempts.

Suicide is the third leading cause of death in 15-19 years old (WHO, 2018) and it was estimated that about 62,000 adolescents died because of self-harm in 2016 (NIHC, 2016). The burden of teen depression is worsened by the difficulties in obtaining diagnosis and treatment for this disorder, especially in low and middle-income countries (LMICs), where adolescents account for 90% of the total adolescent population worldwide (NIHC, 2016). Among the main reasons for under-diagnosis of depression in LMICs are the poor knowledge or awareness about psychiatric disorders among health workers, and the stigma preventing adolescents from seeking help (Wainberg et al., 2017). The burden of depression in LMICs is worsened by the intrinsic

difficulties in large mental health screening and by the poorer equipment available, as well by the social and cultural gap which characterized these poorest nations (Rathod et al., 2017).

The complexity of MDD pathophysiology and its heterogeneity has represented an important challenge for its diagnosis and treatment. However, adolescent depression was proven more challenging for clinicians, and the reasons are multiple. Adolescent and children depression diagnostic criteria are the same used to define MDD in adults, with the exception that irritable rather than depressed mood is allowed by the DSM-IV and 5 as a core diagnostic mood symptom for children and adolescents (APA, 2000, 2013). However, differences in symptomatology were observed between adolescents and adults. For example, vegetative symptoms such as appetite and weight disturbance, fatigue and insomnia are more common in depressed children and adolescents, whereas hypersomnia seems to be less common. Regarding somatic symptoms, musculoskeletal pain and headaches are particularly frequent in teen-agers (Rice et al., 2019). Differences in adolescents' symptomatology can also differ accordingly to the severity of depression, as observed in the study of Cole and colleagues; specifically, concentration problems, feelings of worthlessness and sleep problems are present at low levels of depression severity, whereas psychomotor agitation/retardation, weight and appetite problems and suicide ideation are more common only at high levels of depression severity (D. A. Cole et al., 2011). In the very recent work by Rice and colleagues (Rice et al., 2019), the manifestation of depressive symptoms in adolescents and adults was compared and it was shown that in adolescents loss of energy (97%), insomnia (87%) and appetite and weight changes

(87%) were more common than in depressed adults (71%, 63% and 59% respectively). Interestingly, Rice and colleagues also did not find any evidence that irritability was more common in adolescent than in adult depression, despite irritable mood was allowed to be one of the core symptoms for adolescent depression (Rice et al., 2019).

1.3.1. Genome-wide gene expression studies in adolescent depression

Following the chapter 1.2 on gene expression studies in depression, it is noteworthy to mention that the majority of the studies investigating gene expression in MDD were conducted so far on adult individuals, whereas only a paucity of them on adolescents (Chiang et al., 2019; Ota et al., 2020; Pajer et al., 2012; B. Zhao et al., 2022).

In terms of genome-wide gene expression studies, the very recent study of Zhao and colleagues performed RNA-Seq analysis on peripheral blood samples of ten depressed adolescents and ten controls aged between 14 and 19 years old, yielding almost nineteen thousands of genes differentially expressed (B. Zhao et al., 2022). After performing a functional enrichment analysis of those genes, they identified that those strongly linked to MDD were involved in apoptosis, TNF signalling pathways, and NF-kappa β signalling pathway. Furthermore, a pathways enrichment analysis showed that the pathways most significantly associated with the DEGS were principally involved in positive regulation of histone methylation, positive regulation of T cell mediated immunity, and postsynaptic density. Overall, most of the genes and pathways linked to MDD were associated with stress response and immunity. Therefore, this study supported the role of inflammation, immune response, and neurodegeneration in adolescent depression.

A further study using genome-wide gene expression analysis to investigate the mechanisms underlying adolescent depression was conducted by Chiang and colleagues in 2019, by performing RNA-Seq analysis on PBMCs from peripheral blood samples of 87 adolescents aged 18 years old (Chiang et al., 2019). An up-regulation of inflammation-related genes and a down-regulation of antiviral-related genes was observed in adolescents with depression compared with their non-depressed peers. Specifically, they observed that the pattern of differential gene expression was mediated by an increased activity of the pro-inflammatory NF-Kβ, and by a reduced activity of glucocorticoid receptors and interferon response factors, suggesting again a link between depression and immunity also in adolescent depression.

1.4 Identifying Depression Early in Adolescence (IDEA Project)

The aim of the IDEA consortium was to develop risk detection strategies incorporating biological, psychological, and social factors that can be assessed in different global settings and populations. The ultimate and ambitious goal of the IDEA project was to improve the early detection and prevention of adolescent depression, starting from Brazil and LMICs and extending the findings worldwide. The IDEA project focused its attention on three different cultural and economic settings: Brazil, Nepal, and Nigeria. Brazil is an upper-middle income country that is witnessing a rapid development regarding economics and urbanization. Nepal is one of the poorest countries in the world where many adolescents live in humanitarian settings due to recent emergencies such as civil war and environmental disasters. Nigeria is the most populous LMIC in Africa and it accounts for a large adolescent population that is experiencing the rapid development of African economies, chronic exposure to political and community violence and high rates of infectious disease such as HIV.

In the context of the IDEA project, it is important to acknowledge that about 30% of depressed patients do not respond to first line antidepressant drugs and 20% do not achieve an amelioration on symptomatology even after more than three different antidepressant approaches (Labermaier, Masana, & Muller, 2013). Moreover, the stigma of depression and generally mental health prevents individuals to seek for help, leading to a deterioration of the symptomatology. Frequently, antidepressant drugs carry heavy side effects which leads to abandon the therapy. Lastly, the access to a prompt and proper diagnosis of depression and to initiate the most suitable antidepressant therapy is much more difficult in LMICs, leading to the increase of the

burden of depression in these poorer settings. Thus, predicting and then preventing the onset of depression represents an important step towards reducing the burden associated with adolescent depression and improving the general condition of adolescents with depression. Since depressive symptomatology typically become manifest during adolescence, this was considered as an important temporal window in which act for preventing the onset of disorders by the IDEA team. The need for improving prevention strategies for adolescent depression led to the development of the Identifying Depression Early in Adolescence (IDEA) project, which is a multi-disciplinary global mental health consortium with the main aim to help identification of adolescents at high or low risk of developing depression with a specific focus on LMICs. My doctoral thesis used the IDEA project as main platform for improving our understanding of biological pathways involved in increased risk and development of adolescent depression.

1.4.1 The IDEA Risk Score

Given these premises, as of the main goal of the IDEA Project was to early identify adolescent at risk of developing depression, the IDEA consortium thus developed a composite score (IDEA-Risk Score or IDEA-RS) to estimate individual-level probability of developing MDD among Brazilian adolescents. This composite risk score is a multivariable prediction model developed using data from the population-based 1993 Pelotas Birth Cohort, a prospective study conducted in south Brazil (Rocha et al., 2021). The study included every child born in the city in 1993, resulting in a total cohort of more than five thousand individuals. The data for the IDEA-RS were collected during the assessments at age 15 and 18 and, by using only sociodemographic

variables easily obtainable directly from the adolescent at age 15, a risk calculator was developed to identify those adolescents at risk for developing MDD at age 18. The 11 selected and easily obtainable sociodemographic variables were:

- Biological sex
- Skin color
- Drug use
- School failure
- Social isolation
- Fight involvement
- Poor relationship with mother
- Poor relationship with father
- Poor relationship between parents
- Childhood maltreatment
- Run away from home (Rocha et al., 2021).

The IDEA-RS exhibited good discriminative performance to screen individuals at high and low risk for developing depression at age 18. Specifically, in the Pelotas Birth Cohort the discriminative capacity to distinguish between adolescents who later developed depression or not at age 18 ranged from 0.76 to 0.79 assessed by the C-statistics (Rocha et al., 2021).

Moreover, differently from many other prediction models, which were not externally validated, the Brazilian risk score was validated in other countries such as UK and New Zealand. Firstly, the validation was performed in the Environmental Risk (E-Risk) Longitudinal Twin Study, tracking the development of a nationally representative birth

cohort of 2,232 twins born in England and Wales in 1994-1995. Secondly, the Dunedin cohort was also used for the external validation, which represents a longitudinal investigation of health and behavior in a complete birth cohort born between 1972 and 1973 in Dunedin (New Zealand). When applying the IDEA-RS to these two cohorts, an expected drop of the performance was observed, specifically a C-statistics of 0.59 for the E-Risk and 0.63 for the Dunedin Cohort (Rocha et al., 2021). The lower scores resulted in the external cohort is due to several reasons that indeed does not reduce the positive results obtained. Firstly, the variables from both the independent datasets did not perfectly pair with the eleven variables selected from the Pelotas study; specifically, in the E-Risk dataset 13.1% of the original model information was not available, whereas this loss accounts for 6.9% in the Dunedin cohort. Moreover, although all the three cohorts presented an assessment for depression at age 18, only the Pelotas and the Dunedin cohort presented assessments at age 15, whereas for the E-risk cohort data from the assessment at 12 years old were used. Indeed, the differences in the predictors' availability could have influenced the performance of the IDEA-RS in the other settings.

Noticeably, the ability of the IDEA-RS to predict the future onset of depression in adolescents was subsequently tested in two LMICs located in different continents and with different cultural, social, and economic backgrounds, such as Nepal and Nigeria. In Nepal, this prediction model was applied to data from a longitudinal study of former child soldiers matched with war-affected civilian adolescents. Out of the eleven sociodemographic variables employed by the original IDEA-RS, seven were included in the model (biological sex, ethnicity, drug use, school failure, social isolation, fight involvement and childhood maltreatment), resulting thus in 13,2% of the original

Pelotas model's information lost. The model showed an overall good performance, achieving good discrimination between depressed and non-depressed individual with an area under the curve (AUC) of 0.73 (confidence interval 0.62-0.83) (Brathwaite et al., 2021). Similarly, also in Nigeria the prediction model was reduced from eleven to seven predictors (biological sex, drug use, ran away from home, school failure, fights, childhood maltreatment, and social isolation), and it was able to predict future depression in an adolescent students' sample in Lagos. Specifically, it was estimated a chance of 62% (from 58.1% to 65.7%) that a randomly selected Nigerian adolescent who developed depression would have a higher risk than a randomly selected Nigerian adolescent who did not develop depression (Brathwaite et al., 2020).

Taking together, these results of the external validation might suggest that this prediction model can play a role in an early identification of vulnerable adolescents and that can be used in other LMICs to reduce the burden of depression and allow wider screening in the poorest settings worldwide. Indeed, adjustments of the IDEA-RS must be made accordingly to the different cultural, economic, and social background of the populations to which the risk score is applied. This might represent a limitation, however it is important to acknowledge that different cultural background requires specific attention, as a specific risk factor might not be considered in the same way worldwide.

1.4.2 The IDEA-RS and the IDEA-RiSCo

This IDEA-RS was adopted in the recruitment of a cohort of adolescents at high and low risk of developing depression in the context of the IDEA project in Brazil, named IDEA-Risk Stratified Cohort (IDEA-RiSCo). The IDEA-RiSCo is represented by a new

sample of adolescents screened for low or high risk of developing depression, as well as a group of adolescents with a currently untreated major depressive episode (Kieling et al., 2021). The IDEA-RiSCo is a milestone of my doctoral thesis, as it represents the cohort at the base of all the biological analysis that I conducted and that will be described and presented during this doctoral thesis. Briefly, 150 adolescents aged between 14 and 16 years old were recruited in the city of Porto Alegre, in the south of Brazil, and divided into three groups: i) 50 adolescents classified as having high risk of developing depression, ii) 50 adolescents classified as having low risk of developing depression, and iii) 50 adolescents with a current diagnosis of depression and high risk of developing it accordingly to the IDEA-RS. The detailed recruitment process and further details will be discussed in the Methods section (paragraph 3.1).

1.5 Sex differences in depression

1.5.1 Sex differences – General considerations

Biological sex refers to the biological differences between males and females; the Institute of Medicine defines sex as *"being male or female according to reproductive organs and the functions assigned by chromosomal complement (XX for female and XY for male)"*. On the other hand, gender refers to social and cultural factors related to being a man or a woman, also depending on historical and cultural backgrounds (Wizemann & Pardue, 2001). This premises is necessary as sex and gender were misused in the last decades since they were used as synonyms also in several scientific fields. In this chapter, the term sex is referring to biological sex.

Biological sex plays a major role in the incidence of several disorders (Zonca, 2022b). To give some examples, the number of females suffering from Alzheimer's disease, Huntington's disease and multiple sclerosis were shown to be significantly higher compared with males, whereas males were shown to be more likely to suffer from Parkinson's disease (Ullah et al., 2019). Moreover, also the incidence of mental health related disorders is deeply affected by biological sex, as it was widely observed that females are more likely to suffer from depression, anxiety, panic disorder, phobias and agoraphobia compared with the male counterpart (Bandelow & Michaelis, 2015). Again, sex differences were observed also in developmental disorders, such as Tourette syndrome (90% males), autism spectrum disorder (80% males), attention deficit hyperactivity disorder (80% males), schizophrenia (73% males), anorexia nervosa (93% females) and anxiety disorder (67% females) (Bao & Swaab, 2010).

1.5.2 Sex differences in incidence of depression

Although many psychiatric disorders present a sex-driven unbalance in their incidence as it was previously discussed, depression is indeed one of the mental disorders where this is most evident. The incidence ratio of depression is 2:1 in females compared with males, and this difference is almost the same through the entire lifespan (as previously described for adolescents). However, male children from 2 to 8 years old were shown to be slightly more likely to suffer from depressive episodes compared with girls (Cree et al., 2018), whereas the incidence of depression in females dramatically increases from early adolescence, as depression is more than twice as prevalent in young females aged 14-25 years old than males (Shorey, Ng, & Wong, 2021).

The possible reasons behind this variance in depression between females and males were deeply researched but a generally accepted conclusion has not been reached yet and remain under investigation (Zonca, 2022b). It is noteworthy to consider that the approach toward the symptomatology and how males and females react to depression seems to be opposite directed. Indeed, females are keener to ask for help and to engage in help-seeking behaviors, as they are more prone to seek treatment and psychological help in the earliest stages of the disorder. On the other hand, males are less disposed to ask for help but they engage in different more detrimental copying strategies, such as by increasing alcohol and drug abuse (Frackiewicz, Sramek, & Cutler, 2000). Although it is important to acknowledge differences in the help-seeking behaviors and copying strategies as part of the broader mechanisms in the explanation of sex differences in depression, it would be too simplistic to focus only

on these differences to explain the differences in the incidence of depression across sexes.

Indeed, sex hormones were hypothesized as one of the biological factors that can possibly explain such sex differences in depression. Indeed, changes in hormone levels during puberty, menstruation, pregnancy, and menopause were hypothesized to drive the sex differences in depression (Sramek, Murphy, & Cutler, 2016). For example, hormonal fluctuation during puberty were shown to be associated with an increased risk for developing depression in females (Studd, 2015), and this risk was shown to further increase after the menarche (DelRosario, Chang, & Lee, 2013; Parker & Brotchie, 2010; Studd, 2015). Moreover, both estrogens and progesterone were shown to be involved in mood modulation (Brinton et al., 2008) and higher levels of testosterone were associated with increased number of suicide attempt in females affected by bipolar disorder (Sher et al., 2014).

Together with sex hormones, also immunity was hypothesized to play a role in sex differences in depression, as differences across the two sexes in the immune response were observed. For example, higher inflammatory marker levels, and higher risk of developing autoimmune disorders were observed in females (Quintero, Amador-Patarroyo, Montoya-Ortiz, Rojas-Villarraga, & Anaya, 2012; Yang & Kozloski, 2012). Moreover, worsening of symptoms in various inflammation-related chronic diseases, were observed to be driven by menstrual cycle (Oertelt-Prigione, 2012). On the other hand, male sex hormones were shown to have mainly anti-inflammatory proprieties (Gilliver, 2010), whereas female sex hormones have both pro- and anti-inflammatory proprieties (Bereshchenko, Bruscoli, & Riccardi, 2018).

Taken together, these finding suggested the existence of a possible link between immunity and sex hormones that might explain sex differences in depression; this hypothesis was recently investigated in the meta-analysis of Lombardo and colleagues, that showed a possible protective roles of testosterone and exogenous female sex hormones via their modulation of the immune system (Lombardo, Mondelli, Dazzan, & Pariante, 2021).

There are also some disorders which are naturally related to one sex only, such as post-partum depression. Briefly, post-partum depression accounts for about 20% of the deaths by suicide after giving birth and it represents a serious condition for both the mother and the child. The incidence of post-partum depression ranges from 10% to 25%, and the depressive symptomatology usually lasts more than 6 months among 25% - 50% of females suffering from this condition (Beck, Records, & Rice, 2006). Moreover, another very common mood disturbance in the post-partum period is maternity blues, which is characterized by mild symptoms such as mood lability, irritability, generalized anxiety, sleep and appetite disturbance (Watanabe et al., 2008). As shown by a recent meta-analysis, the incidence of maternity blues is higher than post-partum depression as it affects from 14% to 75% of the new mums (Rezaie-Keikhaie et al., 2020). The mechanisms underlying both post-partum depression and maternity blues are probably attributable to the robust hormone changes that characterize pregnancy and the postpartum period, which persist also during the lactation period. Moreover, together with changes in estrogens and progesterone levels, some alterations were observed also in the HPA axis and in stress response (Brunton, Russell, & Douglas, 2008). Changes in hormone levels during pregnancy

were shown to influence the female brain: by parturition, the maternal brain shrinks by 8% and reverts to its original volume within 6 months postpartum (Oatridge et al., 2002). These changes might be responsible for the increased vulnerability to develop psychiatric symptoms in the postpartum period.

1.5.3 Sex differences in symptomatology of depression

The clinical manifestation of depression differs between males and females, as different depressive symptoms were observed accordingly to biological sex. Starting from adolescence, which is a period of life characterized by intrinsic behavioral and hormonal differences in males and females, adolescent females report to experience more guilt and bodily dissatisfaction, self-disappointment, feelings of failure and concentration problems than males. On the other hand, males experience more anhedonia, morning depressed mood and morning fatigue (Bennett, Ambrosini, Kudes, Metz, & Rabinovich, 2005). Differences across sexes in the symptomatology persists also in adulthood and they were widely investigated in the STAR∗D study; at baseline adult females reported greater rates of hyperphagia and weight gain, hypersomnia, gastrointestinal disturbances as well as were more likely to report anxiety-like behavior and suffer from bulimia (Marcus et al., 2008). On the other hand, adult males reported an increase in comorbid substance abuse and deadly suicide attempts.

Hence, these differences in symptoms might represent the tip of the iceberg of further differences driven by biological sex; understanding and investigating the mechanisms – either biological or not – underpinnings those differences might be of paramount importance for better understanding the pathophysiology of depression.

Besides from depressive symptomatology, differences across sexes were investigated also in relation to the pathways towards MDD, specifically considering how specific risk factors can differently affect males and females. This aspect was investigated in a co-twin study that matched sisters and brothers on genetic and familial-environmental background. This study showed that low parental warmth, parental loss, neuroticisms, lifetime trauma, divorce, social support, and marital satisfaction contributed more significantly to the onset of MDD in females rather than in males. On the other hand, low self-esteem, drug use disorder, history of MDD, and distal and dependent recent stressful life events were more strongly associated with males (Kendler & Gardner, 2014). In line with these evidence, two categories of depression were proposed: the anaclitic and the introjective depression. The first one refers to the vulnerability to interpersonal relationship and it is more common among females, whereas the second is characterized by a vulnerability to low self-esteem and it is more common in males (Reis & Grenyer, 2002). For instance, this was observed in a study investigating differences across sexes with regards to their self-perceived changes on the workplace. Male workers with MDD were observed to report more often reduced work efficiency, whereas depressed females were shown to be more likely worried about deterioration in relationship with colleagues and superior (Niki et al., 2020). Taking together, this evidence suggests that males are more sensitive to failure to achieve goals, whereas females are more sensitive to interpersonal relationship. Thus, depression seems to deeply impact self-esteem and worries regarding financial, work, and legal problems in males, whereas failure in social relationship as well as in marital status and family problems seems be more affected in depressed females.

1.6 Risk factors and biomarkers for adolescent depression

Considering that treatment alone of depression is not sufficient to completely address the problem and to reduce its burden, the identification of those adolescents at risk of developing depression as well as the implementation of preventive strategies can surely represent a milestone in the battle against adolescent depression. To achieve this goal, possible risk factors for depression were widely investigated and identified over the last decades, including biological and environmental risk factors. However, given the well-known fact that depression is an extremely heterogenous condition, identifying just one risk factor responsible for the onset of MDD in an individual is un-realistic. The existence of risk factors is deeply connected with the concept of vulnerability and resilience to depression, which is particularly important for the development of prevention strategies. For example, stress was shown to be an important risk factor for the development of several psychiatric disorders, but its impact on each individual can be different: although experiencing stressful events represents a risk toward the development of mental health problems, not all the exposed individuals will end up developing psychiatric disorders. On the contrary, many acquire coping strategies and become resilient to stress, whereas other might become vulnerable and thus at higher risk of developing psychiatric disorders, such as depression (Pfau & Russo, 2015; Wu et al., 2013).

1.6.1 Risk factors for depression in the context of the IDEA project

In the recent literature, several studies focused on prevention and early detection of adolescent depression; however, these studies were all primary conducted in westernized, educated, industrialized, rich and democratic setting – the so-called

WEIRD societies- with a large gap for studies conducted in LMICs. Unfortunately, this gap represents a significant problem since all the strategies that were applied to prevent mental health disorders in LMICs were based on concepts and risk factors which were developed by assumptions from WEIRD populations. Accordingly to the critical analysis that were reported also by the IDEA consortium, using risk factors from HICs to be applied to LMICs is problematic mainly for three reasons: 1) risk factors may be interpret differently across different cultural context; 2) risk factors may have different impact across different populations; 3) the feasibility of assessing and measuring specific risk factors varies across different settings (Wahid et al., 2021). Sign and symptoms of depression as well as risk and protective factors can manifest differently across settings, specifically among HICs and LMICs which are extremely different in terms of culture, economy, environment, family structures, gender role, and societal norms. Hence, there is a need for a context-specific understanding of mental health, adolescent experience and risk and protective factors in LMICs, as cultural conceptions of depression were shown to significantly vary across context and populations. Moreover, the identification of screening tools in LMICs might be difficult due to the actual feasibility of applying such preventive strategies in different settings; hence, assessing the feasibility of such research is a necessary step to be considered. Lastly, LMICs usually lack of specific ethical and institution policies for research involving adolescents, specifically when considering psychiatry (Wahid et al., 2021).

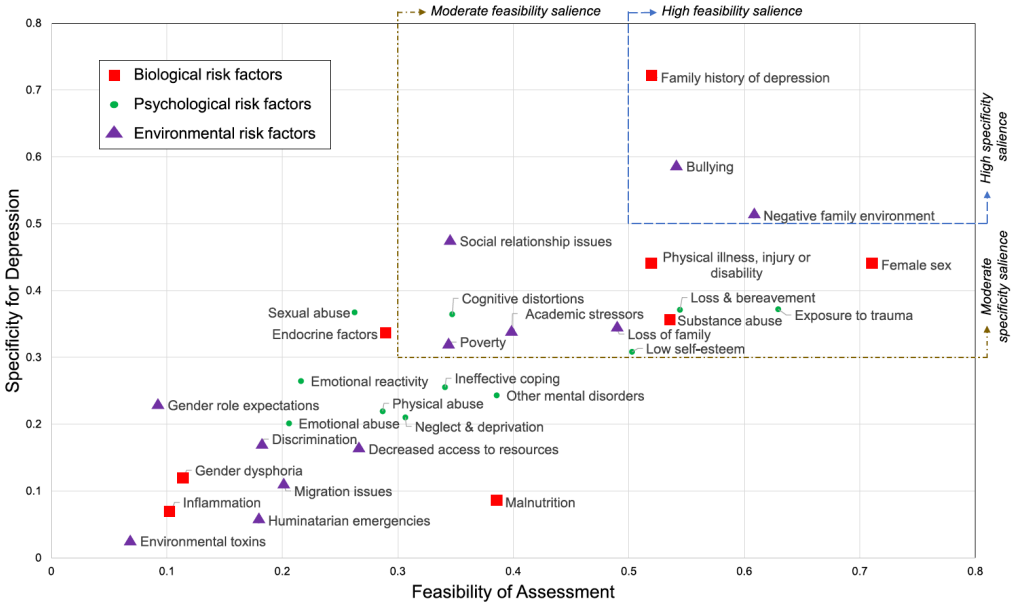
The aim of understanding the feasibility of assessing risk factors in adolescents from diverse cultural, social and economic settings (such as LMICs) and which might be those risk factors is one of the points which were addressed by the IDEA consortium by a Delphi study, which is a consensus building approach that consists in collecting

expert opinion through multiple survey panels, and which is able to fill the literature's gap by providing a "state of the field" expert recommendation as base for further action. In the IDEA Delphi study, the experts were requested to identify biological, psychological, and environmental risk factors for adolescent depression as well as providing feedback regarding early signs and detection strategies of adolescent depression (Wahid et al., 2021). When addressing biological risk factors, the most highly ranked were female sex, substance abuse, physical illness/injury or disability, and family history (Figure 1.2). Regarding psychological risk factors, they identified exposure to trauma, loss and bereavement, sexual abuse, cognitive distortion, and self-esteem. Lastly, the most highly ranked of the environmental risk factors were bullying, family environment, social relationship issue, loss of family, academic stressors, and poverty. Among those risk factors, three were ranked as highly specific and highly feasible to measure in LMICs: family history of depression, exposure to bullying and negative family environment; six were considered as modestly specific but highly feasibly to measure: physical illness or disability, female sex, bereavement, trauma exposure, substance abuse and low self-esteem. Five of them were ranked as modestly specific and modestly feasible: social difficulties, academic stress, poverty, loss of family and cognitive distortion. Lastly, five were ranked as less modestly specific and feasible to measure: mood changes, loss of interest, social isolation, suicidality, and sleep changes (Wahid et al., 2021). It is noteworthy to mention that inflammation was mentioned and included in the biological risk factors, suggesting the knowledge and the concern about it, but it was not ranked as one of the most significant risk factors, indicating a critical skepticism towards the feasibility of using inflammation as a risk factor for screening adolescent depression in LMICs, and

suggesting also the differences between HICs and LMICs settings in terms of choosing the most suitable and feasible panel of risk factors (Wahid et al., 2021).

Given these premises, the following chapter will provide a short overview of the environmental and biological risk factors for the onset of depression by considering the current literature, which considers mainly cohorts from HICs.

Figure 1.2. Biological, psychological, and environmental risk factors from the IDEA Delphi study Y-axis: higher scores represent greater ranking of specificity for adolescent depression; X-axis: higher scores represent greater ranking for ease of feasibility to assess the risk factor in the respondents' context. Figure from (Wahid et al., 2021).



1.6.2 Environmental risk factors

Familial history of depression and/or mental health disorders were associated to increased risk of developing depression. Children born from parents with depression are three to four times more likely to face depression than children of parents without MDD (Weissman et al., 2006). Moreover, familial depression was shown to impact also the third generation affecting around 60% of grandchildren (Weissman et al., 2005). In addition, children of parents with depression are more likely to experience more severe and continuous courses of depression (Goodman et al., 2011). Although it was deeply shown that children of parents with depression are at heightened risk for developing MDD, yet little is known about the specific mechanisms underlying this risk. The model of the transgenerational transmission of depression was proposed by Goodman and Gotlib, and it hypothesized four possible mechanisms: 1) heritability, 2) innate dysfunctional neuro-regulatory factors, 3) negative maternal cognitions, behaviors, and affect, and 4) stressful context of the children's life, which are linked to cognitive, affective and behavioral deficits in children (Goodman & Gotlib, 1999). The study of Loechner and colleagues compared various potential mechanisms and it showed that children of parents with depression presented significantly more symptoms of depression and general psychopathology, less adaptive emotion regulation strategies, fewer positive life events and fewer positive parenting strategies compared with children of parents without MDD (Loechner et al., 2020). Familiar depression was shown to be associated with younger age of onset of MDD, specifically in patients with depression onset before age 20 (Kendler, Gatz, Gardner, & Pedersen, 2005; Tozzi et al., 2008).

Another important environmental risk factor acknowledged by the recent literature is the exposure to early life stress, which was shown to be a vulnerability factor for the onset of depression through the entire lifespan (LeMoult et al., 2020; Mandelli, Petrelli, & Serretti, 2015; Widom, Dutton, Czaja, & DuMont, 2005). A European Report from WHO indicates that at least 18 million children in Europe suffer from childhood trauma, harming mental and physical health. According to the Centers for Disease Control and Prevention in USA, childhood maltreatment is defined as "*acts of commission or omission by parents or other caregivers (e.g., clergy, coach, teacher) resulting in potential harm to the child's health*". Acts of commission are divided into physical, sexual, and psychological abuse, whereas acts of omission are classified as physical, emotional, medical, or educational neglect or failure to supervise (Hauser, Schmutzer, Brahler, & Glaesmer, 2011). Several studies in literature showed that the onset of mood disorders, such as depression, was deeply influenced by stressful life events experienced in childhood (Horesh, Klomek, & Apter, 2008; K. S. Kendler, J. Kuhn, & C. A. Prescott, 2004a; K. S. Kendler, J. W. Kuhn, & C. A. Prescott, 2004b). In one community-based study of approximately 2,000 women, those with a history of childhood physical or sexual abuse had an increased risk of depression and anxiety and were more likely to have attempted suicide than women without such a history (Kendler et al., 2004b).

1.6.3 Biological risk factors

Biological markers were deeply investigated with the aim to better guide and develop interventions and prevention strategies for depression (Lombardo et al., 2019; Raison & Miller, 2013). Although various biological mechanisms were explored and

examined, no biomarkers were clearly identified or validated for risk or presence of depression in adolescence and young adulthood (D'Acunto, Nageye, Zhang, Masi, & Cortese, 2019; Lopez-Duran, Kovacs, & George, 2009). Several theories explaining biological pathways underpinning depression in adults were proposed, including the monoamine theory, the increased activation of the immune system, and abnormalities of the HPA axis (Dowlati et al., 2010; Kennis et al., 2020; Pariante & Lightman, 2008). Other potential biomarkers were suggested based on neuroimaging studies (e.g., fronto-limbic dysregulation with hyperactivity in limbic brain structures and hypoactivity in the prefrontal cortex) (Jaworska, Yang, Knott, & MacQueen, 2015; Zhong et al., 2011). Other associated biomarkers included endocannabinoids (Hill, Miller, Ho, Gorzalka, & Hillard, 2008; Z. E. Zajkowska, Englund, & Zunszain, 2014), neurotrophic factors (Duman & Li, 2012), polyunsaturated fatty acids (PUFAs) (Grosso et al., 2014), hormones (Hacimusalar & Esel, 2018), telomere length (Simon et al., 2015), and vitamin D (Menon, Kar, Suthar, & Nebhinani, 2020), but these associations lack consistency across studies and populations.

1.6.3.1 Inflammation

Inflammatory mechanisms were suggested to play an important role in the pathogenesis of depression. As shown by a series of recent meta-analysis, higher levels of peripheral inflammatory cytokines were widely observed in adult patients with depression (Kohler et al., 2017; Leighton et al., 2018; Osimo, Baxter, Lewis, Jones, & Khandaker, 2019; Osimo, Pillinger, et al., 2020). Individuals with MDD showed higher serum or plasma levels of pro-inflammatory cytokines, such as IL-6, IL-10, IL-12, IL-13, IL-18, and TNF-α, compared with healthy controls (Himmerich, Patsalos,

Lichtblau, Ibrahim, & Dalton, 2019); moreover, the hyperactivation of the immune system were considered as a risk factor for the development of depression (Raison, Capuron, & Miller, 2006). Increased levels of inflammatory markers were also associated with reduced response to antidepressant medications. The recent meta-analysis of Liu and colleagues showed reduced baseline levels of IL-8 and CRP in patients with depression who subsequently respond to antidepressant drugs (J. J. Liu et al., 2020), and Cattaneo and colleagues showed evidence of inflammasome activation and glucocorticoid resistance in both drug-free and TRD patients with depression (Cattaneo et al., 2020).

Far less studies focused their attention on adolescent depression. The meta-analysis of D'Acunto and colleagues reported higher TNF-α levels in adolescents aged up to 18 years old with depressive disorders versus control subjects (D'Acunto et al., 2019). Similarly, the meta-analysis of Colasanto and colleagues showed higher levels of CRP and IL-6 in adolescents with depression compared with their control peers (Colasanto, Madigan, & Korczak, 2020). Adolescent girls at risk of developing depression due to family history or cognitive vulnerability, were shown to have elevated CRP at a 6-month follow-up, and that high levels of IL-6 were associated with increased depression risk at follow-up (G. E. Miller & Cole, 2012). Similar results were also reported by Khandaker and colleagues, showing high levels of IL-6 in association with greater depression risk (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014). Lastly, Moriarity and colleagues observed that increases in TNF-α predicted increase in depressive symptoms, whereas CRP, IL-6, IL-8, and IL-10 did not have significant within-person effects on change in total depressive symptoms (Moriarity et al., 2020).

1.6.3.2 HPA axis

Given the already discussed link between stress and depression, researchers focused their attention on HPA axis activity by measuring cortisol levels in saliva and blood samples of adolescents, as possible biological mechanisms involved in the presence or risk for adolescent depression. However, current literature reported heterogenous results. For example, elevated morning cortisol levels were linked to increased vulnerability in developing depression during adolescence (Adam et al., 2010; Owens et al., 2014; Vrshek-Schallhorn et al., 2013). Conversely, blunted cortisol response to a laboratory stressor was associated with subsequent increase in depressive symptoms (Keenan et al., 2013). A possible explanation of such inconsistencies might be the different developmental stages at which participants were assessed across the studies, as it is known that pubertal development is characterized by changes in cortisol production (Colich, Kircanski, Foland-Ross, & Gotlib, 2015).

Furthermore, the role of cortisol in the development of adolescent depression was investigated in the very recent meta-analysis published by our group in the context of the IDEA project. This study investigated the relationship between cortisol and MDD in adolescents and youth aged between 10 to 24 years old. The results showed that elevated morning cortisol levels were prospectively associated with later onset of MDD in adolescent. On the other hand, no differences in morning and afternoon cortisol levels as well as in cortisol stress response were observed between adolescents with depression and controls (Z. Zajkowska, Gullett, et al., 2021). Given these results, we suggested that elevated morning and nocturnal cortisol might represent risk factors for depression in adolescence, supporting the hypothesis of the

hyperactivity of the HPA axis in the development of adolescent depression (Z. Zajkowska, Gullett, et al., 2021).

It is also noteworthy to mention the "pubertal stress recalibration" theory proposed by Gunnar and colleagues (DePasquale, Donzella, & Gunnar, 2019; Gunnar, DePasquale, Reid, Donzella, & Miller, 2019). During infancy the HPA axis can calibrate different environmental conditions, and Gunnar and colleagues suggested that a similar pattern of calibration might occur also during puberty, that it is believed to be a second window of plasticity during which the HPA axis could recalibrate. This hypothesis might give further explanation of the heterogeneity of the results of the studies on HPA axis deregulation as a risk factor of adolescent depression.

1.6.3.3 Interaction between environmental and biological risk factors

A growing body of literature investigated the interaction between environmental and biological risk factors in relation to the onset of depression, both in adolescence and adulthood. Among the environmental risk factors, exposure to early life stress was widely shown to represent a vulnerability factor for the onset of depression through the entire lifespan (Mandelli et al., 2015; Widom et al., 2005; LeMoult et al., 2020). As previously mentioned, the very recent systematic review published by our group in the context of the IDEA project, focusing on adolescents in both HICs and LMICs, showed the association of both environmental and biological risk factors for the onset of depression. Specifically, we found that increased inflammation, telomere length and brain abnormalities (such as blunted reward-related activity, white matter disruptions, and altered volume of limbic brain regions), were associated with increased risk for depression mainly in the context of early life adversity (Z. Zajkowska,

Walsh, et al., 2021). These results supported the importance of the interaction of several biological risk factors, including high inflammation, with the experience of childhood trauma in increasing the risk for future depression among adolescents (Z. Zajkowska, Walsh, et al., 2021).

Moreover, it was previously observed that different sub-types of childhood trauma could differently mediate the risk of developing depression, and the effects of different early in life trauma differed between adolescents and adults (Infurna et al., 2016; Shapero et al., 2014). The recent systematic review of Gill and colleagues, which investigated the association between inflammation in adults with MDD with or without an history of early in life stress, showed increase IL-6 levels in patients with MDD who experience childhood trauma compared with those who did not experiences early in life stress (Gill et al., 2020).

Hence, it is reasonable to believe that considering multiple risk factors, such as environmental risk factors (e.g., childhood trauma) and biological markers (e.g., inflammation) might provide a higher predictive power as well as might help in the development of prevention strategies to modify trajectories from the experience of early adversities to development of depression during adolescence and adulthood.

2. Aims and Hypotheses of the study

Omic approaches such as the genome-wide gene expression analysis were widely used in research for investigating biological mechanisms associated with depression as well as to identify potential peripheral biomarkers (Mariani et al., 2021).

Although many studies used candidate-gene approaches and investigated the expression levels of specific genes, there are also studies which used hypothesis-free approaches.

In the current thesis project, I do not focus on specific genes or biological pathways, but I use omics approaches, specifically microarray and RNA sequencing (RNA-Seq), to discover genes or pathways that are associated with presence or increased risk of depression in adolescents.

The rationale behind the choice of performing a transcriptomic approach rather than investigating for example the epigenome or proteome is mainly because, together with the IDEA team, we firstly aimed to investigate the modulation of gene expression for the identification of pathways possibly associated with adolescent depression. Moreover, given the fact that one of the ultimate goals of the IDEA project was to identify such pathways and genes associated with risk and presence of depression and replicate the findings specifically in LMICs (such as Nepal and Nigeria), transcriptomic seemed more feasible and easier to be translated also in LMICs settings. However, we do acknowledge the importance of other omics techniques, such as the epigenetic and the proteomic.

Microarrays and RNA-Seq are two different omics approaches that focus their attention on the transcriptome. However, they do focus on the same goal in different

ways, as microarrays methods measure the intensity of fluorescence and it is based on a hybridization process, whereas RNA-Seq measures read counts as associated relative abundance measure for gene expression. Both techniques are hypothesis-free, meaning that they are not biased by previous hypothesis of targeted molecules or mechanisms to be researched, but it interrogates the entire transcriptome.

In this doctoral thesis and in the IDEA project, we decided to perform both -omics approaches because the microarray technique is a well-known and established genome-wide gene expression technique, which has been widely used in literature as well as in our research group. On the other hand, RNA-Seq is a novel and promising technique, that is starting to be widely used also in the field of mental disorders. Lastly, using two different techniques will allow us to identify the best one to be used in the future studies, specifically in LMICs in the context of the IDEA project, considering the feasibility as well as the pros and cons of each of them.

2.1 First Aim: Identify biological pathways associated with presence of depression and/or increased risk of adolescent depression by using Microarray technique (Affymetrix)

The Affymetrix Gene Atlas platform is based on the microarray technique, a well-established technique that was widely used in the field of biological psychiatry (Hennings et al., 2015; Woo, Lim, Myung, Kim, & Lee, 2018) as well as in my research group (Cattaneo et al., 2019; Cattaneo et al., 2018; Hepgul et al., 2016; Lopizzo et al., 2021). As a hypothesis-free method, microarray technique is not biased by previous

hypothesis of targeted molecules or mechanisms to be researched, but it interrogates the entire transcriptome. By exploring the whole transcriptome, it allows the investigation of thousands of genes at the same time.

Hence, by using a hypothesis-free approach, I do not make any *a priori* hypothesis regarding specific genes or biological pathways that will be differently modulated in the three groups as a results of the microarray Affymetrix analysis.

However, I hypothesize that different biological signatures are associated with the presence of depression and/or with the risk of developing the disorder in adolescents, and thus different genes and biological pathways will be differently modulated in adolescents with depression or with high or low risk of developing MDD.

2.2 Second Aim: Identify differences between males and females in the biological pathways associated with presence of depression and/or increased risk of adolescent depression by using Microarray technique (Affymetrix)

It is well known that the incidence of depression differs in males versus females as adolescent females suffer from depression twice than their males' peers (Shorey et al., 2021). Given the differences in the incidence and symptomatology of adolescent depression, my hypothesis is that different biological signatures will be driven by biological sex. For this reason, I aim to compare the expression profile across groups by considering male and females separately, allowing the identification of different genes and biological pathways specifically in males and females.

2.3 Third Aim: Identify biological pathways associated with presence of depression and/or increased risk of adolescent depression by using RNA Sequencing

Another omics-based approach that I apply in my PhD project is the RNA Sequencing technique. The same considerations around omics-based approaches previously made for microarrays are still valid for the RNA Sequencing. However, RNA-Seq differs from microarrays as the former allows for full sequencing of the whole transcriptome while the latter only profiles predefined transcripts and genes through hybridization. Thus, RNA-Seq can help identifying more differently modulated transcripts than microarray.

Similarly to what previously described for the microarray technique, since RNA-Seq is an hypothesis-free approach, I do not make any *a priori* hypothesis regarding specific genes or biological pathways differently modulated in the three groups as a result of the RNA-Seq technique.

However, similarly to the first aim, I hypothesize that different biological signatures are associated with the presence of depression and/or with the risk of developing the disorder in adolescents, and thus different genes and biological pathways will be differently modulated in adolescents with depression or with high or low risk of developing MDD.

2.4 Fourth Aim: Identify differences between males and females in the biological pathways associated with presence of depression and/or increased risk of adolescent depression by using RNA Sequencing

As previously discussed for the second aim, I will perform the same comparison for the RNA-Seq analysis among groups by considering males and females separately, as I hypothesize that different biological signatures associated with presence and/or risk of depression will be influenced by biological sex.

2.5 My Contribution

2.5.1 IDEA RiSCo recruitment

The IDEA RiSCo school screening and recruitment in Brazil was performed by the Brazilian team led by one of the principal investigators of the IDEA project, Dr. Christian Kieling. The recruiting team was represented by Pedro Manfro, Rivka Pereira, Anna Viduani, Lucas Battel, Silvia Benetti, Thais Martini, Sandra Petresco, Jader Piccin, Thiago Rocha, Luis Augusto Rohde, Fernanda Rohrsetzer, Laila Souza, and Bruna Velazquez.

I contributed to this part of the project by redacting the standard operating procedure (SOP) for the biological sample collection, specifically PAXgene blood tubes and Oragene saliva samples (saliva samples were collected but are not part of this doctoral thesis). I was also in charge of redacting a troubleshooting guide for biological samples collection and I was the contact person for any doubts regarding the collection, handling, storage, and shipment of biological samples.

2.5.2 RNA extraction from blood samples and RNA quality control

I performed the RNA extraction from all the 150 PAXgene blood tubes by using facilities available at the Laboratories of the IRCCS Centro San Giovanni di Dio Fatebenefratelli, in Brescia (Italy). Before the RNA extraction, I randomized the PAXgene tubes to avoid batch effects during the nucleic acid extraction process. After RNA isolation, I performed the quality control assessment by using both Nanodrop spectrophotometer and Agilent Bioanalyzer 2100. I also visualized and checked the outputs of the quality control analysis for all the samples, and I ran further quality checks for those samples whose RIN was undetectable. After performing the quality check, the samples were stored at -80C for subsequent analyses.

2.5.3 Genome-wide gene expression analysis on blood – Affymetrix Gene Atlas platform

I performed all the steps of the pre-processing, quality control, hybridization on array strips, imaging, and final quality checks for all the 150 samples for the microarray analysis on the Affymetrix Gene Atlas platform. The microarray experiment was performed at the IRCCS San Giovanni di Dio Fatebenefratelli in Brescia.

2.5.4 Biostatistical analysis of Affymetrix raw data

I performed the quality check of the Affymetrix raw data (CEL file) as well as the biostatistical analysis to identify genes differently expressed among MDD, HR and LR groups by using the Partek Genomic Suite Software. I also performed the subsequent pathways analysis by using the Ingenuity Pathways Analysis Software and proceeded with the interpretation of the results from the microarray biostatistical analysis.

2.5.5 Genome-wide gene expression analysis on blood – RNA Sequencing on NextSeq 550

As it was the first time I was approaching myself to the RNA-Seq technique, before starting the processing of the IDEA RNA samples, I spent four months in optimizing the protocol of the RNA-Seq with the support of the Illumina scientific and technical support, of my colleagues Nicola Lopizzo, Luca Sforzini and Dr Veronica Begni, and with the supervision of my second supervisor Dr Annamaria Cattaneo. I then performed the RNA sequencing of all the 150 samples, as well as the quality control of the library before proceeding in the sequencing on the NextSeq 550. The entire RNA-Seq protocol, covering the different steps from the library preparation to the sequencing, was performed by using the NextSeq 550 instrument available at the Department of Pharmacological and Biomolecular Science at the University of Milan.

2.5.6 Biostatistical analysis of RNA Sequencing raw data

In order to acquire skills and expertise in the RNA-seq data analyses, I spent six months in optimizing a suitable pipeline for analyzing RNA-Seq raw data (FASTQ). I was directly involved in this process – together with my colleagues Dr Moira Marizzoni and Nicola Lopizzo and supervised by my second supervisor Dr Annamaria Cattaneo - by conducting a literature search of the state-of-the-art knowledge for RNA-Seq biostatistical analysis. Moreover, I used different software as part of the identification of the most suitable pipeline. Dr Moira Marizzoni optimized the definitive pipeline that was used for analyzing the FASTQ raw data of this project and helped me in performing the biostatistical analyses that included the quality control of FASTQ by using the

FASTQC Software, the quantification of the raw reads by using Salmon and the subsequent transcript-level differential expression analysis by using DeSeq2.

With the contribution of Dr Moira Marizzoni, I checked the quality of the FASTQ and discussed the biostatistical analysis to obtain lists of genes differently expressed among MDD, HR and LR groups. Then, I personally performed the pathways analysis by using the Ingenuity Pathways Analysis Software and proceeded with the interpretation of the results.

3. Methods

3.1 IDEA (Identify Depression Early in Adolescence) Project sample recruitment

3.1.1 Ethical approval

The study was approved by the Brazilian National Ethics Committee, project number 50473015.9.0000.5327, the Hospital de Clinicas de Porto Alegre's Ethics Committee, project number 16-0131 and the King's College's Institutional Research Board, project number LRS-17/18-8327.

Adolescents and their primary caregivers provided written consent prior to entering the study. Approval for the school screening phase was obtained from the 1st Regional Education Bureau, in charge of public state schools in the city of Porto Alegre. All participants received feedback with findings from the diagnostic assessment and were referred for care to the Brazilian public health system if clinically indicated. Situations of imminent risk of self-harm or maltreatment were referred to emergency care or protective services following what required by Brazilian legislation. Participants received no financial incentive for taking part in the study, however they were reimbursed for expenses related to their participation (e.g., travel).

The recruitment of participants was conducted in 4 stages (Table 3.1): school screening, telephone interview, clinical assessment, and biological sample collection (Kieling et al., 2021).

Table 3.1. Criteria for the IDEA Risk Stratified Cohort sample composition

Phase	Inclusion criteria	Exclusion criteria
School screening	Enrolment in 8th to 11th grades Age 14 to 16 years Right-handedness	Absent from school on the day of both assessments Inability to complete the screening questionnaire
Phone invitation	Completed school questionnaire	Metallic accessories Clinical conditions* Use of psychotropic medication over last 30 days Use of anti-inflammatory medication over last 14 days
Clinical interview	IQ > 70 Post-pubertal status	Current or lifetime: Bipolar disorder Schizophrenia or a primary psychotic disorder Autism spectrum disorder Substance use disorder Eating disorder Post-traumatic stress disorder

**Excluded clinical conditions: known brain malformations, epilepsy, recent traumatic brain injury, diabetes, cystic fibrosis, HIV, asthma, rheumatologic conditions such as rheumatoid arthritis, systemic lupus erythematosus, psoriasis, purpura, oncologic conditions such as cancer, lymphoma, leukemia, and severe neurodevelopmental disorders, any recent/active infection or active inflammatory process.*

3.1.2 School screening

For the school screening the only inclusion criterion was being aged 14 to 16 years old. The Brazilian recruiting team, led by Dr Christian Kieling, first contacted the Department of Education from Porto Alegre to get permission to contact school representatives. Then, they arranged with schools for eligible students to take part to the recruitment.

The recruiting team used a screening questionnaire, which was divided in two sequential parts: an open questionnaire with a brief identification form and questions on social media usage and physical activity; and a confidential questionnaire comprised of the Patient Health Questionnaire for Adolescents (PHQ-A), questions on drug use and fight involvement, parental relationship and seven dichotomous questions on lifetime sexual, emotional, or physical maltreatment experiences.

The PHQ-A is an adapted version from the PHQ-9 to be specifically used with adolescents as a screening tool in both clinical (Johnson, Harris, Spitzer, & Williams, 2002) and research settings (Allgaier et al., 2012). It consists of 9 questions with Likert-type response options ranging from “none”, “several days” and “more than half the days”. Each of the 9 items are designed to represent the nine DSM-5 criteria for a Major Depressive episode. The scale was translated from English to Brazilian Portuguese by the research team.

3.1.2.1 Risk Score

The composite IDEA risk score (IDEA-RS) developed by the IDEA consortium was used for the risk-stratification of the adolescent sample (Rocha et al., 2021). This risk score does not consider previous depressive symptomatology as a risk factor for MDD, but

information that could be easily obtained from the adolescents to increase its feasibility use in wider scenarios. The risk score was built up by using a short questionnaire that comprises the following variables:

1. Biological Sex
2. Skin colour
3. Drug use
4. School failure
5. Social Isolation
6. Fight Involvement
7. Poor relationship with the mother
8. Poor relationship with the father
9. Poor relationship between parents
10. Childhood Maltreatment
11. Ran away from home

The lifetime maltreatment experiences were divided into three categories, accordingly to the previous study of Rocha and colleagues (Rocha, Graeff-Martins, Kieling, & Rohde, 2015): no maltreatment (no positive answer), probable maltreatment (one positive answer), and severe maltreatment (two or more positive answers). The predictive risk score presented different percentile cut-offs for males and females, due to the discrepancy in prevalence across sexes.

Administration of the IDEA-RS questionnaire in the schools was performed using a coded, unidentified form distributed to students after information on name, date of

birth, self-reported sex, self-reported race/skin colour, handedness, and parental contact information were collected. Questions were selected to match the original phrasing used in the Pelotas 1993 Birth Cohort study. On average, less than 15 minutes were required for administration of the IDEA-RS questionnaire. Students were allowed to ask clarification questions, but researchers were not allowed to review the form to check for completion (forms were considered to be “complete” and therefore valid when only one answer was provided for each question and all questions were answered). The questionnaire is reported in table 3.2 (Kieling et al., 2021).

Table 3.2. The Identifying Depression Early in Adolescence Risk Score (IDEA-RS) From (Kieling et al., 2021).

** Self-reported skin colour following Brazilian official census categories. For analyses, two categories (white vs. non-white) were formed.*

*** Questions about any lifetime use of alcohol, tobacco, cannabis, cocaine, and inhalants were combined into one variable using the OR rule, generating a binary variable for analyses.*

**** Responses to seven dichotomous questions regarding lifetime psychological, physical, and sexual abuse and/or neglect were combined into three categories: zero positive answers=none, 1 positive=probable, 2 or more answers=severe.*

Sex:	Male/Female
Your skin colour or race is:*	White/Yellow/Indigenous/ Brown/Black
Do you meet your friends often to talk, play or do anything else?	No/yes
Have you ever failed a school grade?	No/yes
Have you ever run away from home?	No/yes
Have you ever tried cigarettes?**	No/yes
Have you ever tried alcohol?**	No/yes
Have you ever tried sniffing glue?**	No/yes
Have you ever tried sniffing solvents or ethyl chloride (EC)?**	No/yes
Have you ever tried marijuana?**	No/yes
Have you ever tried cocaine or crack?**	No/yes
Have you ever tried LSD or acid?**	No/yes
Have you ever tried ecstasy or molly?**	No/yes
Have you ever used weight loss pills?**	No/yes

	Have you ever used tranquilizers or sleeping pills?**	No/yes
	Have you ever used any drug? **	No/yes
	In the last year, did you get into any fight in which somebody got hurt?	No/yes
	Would you say your relationship with your father is:	Great/Very good/ Good/Regular/Bad
	Would you say your relationship with your mother is:	Great/Very good/ Good/Regular/Bad
	Would you say the relationship between your father and mother is:	Great/Very good/ Good/Regular/Bad
	Have you ever been separated from your parents so that you had to stay with someone else?***	No/yes
	At home, have you witnessed fights with physical aggression between adults, or has any adult assaulted a child or teenager?***	No/yes
	Have you experienced not having enough food at home, or have you had to wear dirty or torn clothes because you had no other?***	No/yes
	Have you ever thought or felt that your parents wished you were never born?***	No/yes
	Have you ever thought or felt that someone in your family hated you?***	No/yes
	Have you ever been beaten by an adult in your family or by someone who was taking care hard enough to leave marks or hurt you?***	No/yes

Has anyone ever tried to touch you in a sexual way, or tried to make you touch them against your will, threatening you or hurting you?***	No/yes

Using cut-offs for the IDEA-RS based on the Pelotas 1993 Birth Cohort Study (Rocha et al., 2021), eligible participants were *a priori* stratified into two different cohorts:

A. Low-risk (LR) adolescents: those scoring equal to or below the 20th percentile of the IDEA-RS;
B. High-risk (HR) adolescents: those scoring equal to or above the 90th percentile of the IDEA-RS.

A larger stratum in the LR group compared the HR group was allowed as the absolute risk difference between the 10th and the 20th percentiles was minimal. Moreover, a third group of adolescents with MDD was recruited. To allow for two-by-two comparisons between groups, the adolescents in the MDD group were also required to be classified as HR risk, so with a score equal to or above the 90th percentile of the IDEA-RS.

To optimize the recruitment process and increase the probability that diagnostic criteria for depression were met in the MDD group, but not in the LR and HR groups, during the school screening adolescents also completed the Patient Health Questionnaire—adolescent version (PHQ-A) (Johnson et al., 2002). Specifically, adolescents with a PHQ-A ≤ 6 were considered for further assessment for the LR/HR groups, whereas adolescents with a PHQ-A ≥ 10 for the MDD group.

Overall, by combining the risk prediction score and the total PHQ-A score, the participants were divided in three eligible groups for clinical assessment invitation:

A. High risk (HR): PHQ-A score lower than or equal to 6 AND risk score above the 90th percentile;

B. Low risk (LR): PHQ-A score lower than or equal to 6 AND risk score below the 20th percentile;

C. Depression group (MDD): PHQ-A score higher than or equal to 10, as suggested in the literature and risk score above the 90th percentile of risk.

3.1.3 Telephone interview

After participants were divided in the three eligible groups, the recruiting team contacted them via a telephone interview to assess possible exclusion criteria and schedule the clinical assessment. These exclusion criteria applied were:

- current (i.e., in the last two weeks) use of psychotropics, antibiotics, beta-blockers or anti-inflammatory medication;
- presence of metallic implants (dental braces, piercings, pacemaker, etc.);
- current active infection;
- presence of clinical comorbidities requiring current treatment (HIV, asthma, epilepsy, brain malformations, diabetes, cystic fibrosis, etc.);
- history of cranioencephalic trauma or concussions; pregnancy; and recent (i.e., past 3 months) tattoos.

If adolescents were willing to participate, an appointment was scheduled and adolescents were instructed to be fasting, not smoking, or drinking alcohol, avoiding strenuous physical activity, and not interrupting any continuous non-excludent medication (e.g., oral contraceptives).

3.1.4 Clinical assessment

After the school screening, adolescents meeting the criteria for further assessments were evaluated by trained psychologists for cognitive measurements and by skilled child psychiatrists for clinical diagnoses. Before proceeding with the clinical assessment, the child psychiatrist reassessed the telephone interview exclusion criteria, especially for recent use of medications. Absence of a lifetime history of depression for the HR and LR groups was assessed using the Brazilian Portuguese translation of the Schedule for Affective Disorders and Schizophrenia for School-age Children-Present and Lifetime Version (K-SADS-PL) by trained clinicians. Participants in all three groups were excluded if they met lifetime diagnostic criteria for autism spectrum disorder, bipolar disorder, eating disorders, post-traumatic stress disorder, schizophrenia, or substance use disorders.

Youth assigned to LR, HR and MDD group underwent phenotypic assessment; psychological and socio-environmental assessments including self- and clinician-based are detailed in table 3.3 and are listed below (only those relevant for the recruitment are described), for both adolescents and primary caregivers.

- DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure—Child Age 11–17

The DSM-5 Cross-Cutting symptom scale is a self-report instrument for the assessment of twelve psychiatric constructs over the last two weeks including depressive symptomatology, anger, irritability, mania, anxiety, somatic symptoms, inattention, suicidal ideation and attempts, psychosis, sleep troubles, repetitive thought and behaviours, and substance use (Bastiaens & Galus, 2018).

- Wechsler Abbreviated Scale of Intelligence (WASI)
- Parental Bonding Instrument (PBI)

- Self-Administered Physical Activity Checklist
- Mood and Feelings Questionnaire (MFQ)
- Childhood Trauma Questionnaire (CTQ)

The CTQ is a self-report measure of traumatic experiences consisting of 28 items that assess 5 dimensions: physical abuse and neglect, emotional abuse and neglect and sexual abuse.

- Snaith-Hamilton Pleasure Scale (SHAPS)
- Reflective Functioning Questionnaire for Youths (RFQY)
- Affective Reactivity Index (ARI)
- Mood Disorder Questionnaire (MDQ)
- Spence Children's Anxiety Scale (SCAS)
- The Youth Strength Inventory – Adolescent version (YSI-A)
- Adolescent Resilience Scale (ARS)
- U–Change Home Questionnaire Pack
- Tanner Puberty Staging Scale
- Brazilian Economic Classification Criteria (ABEP)
- Kiddie – Schedule for Affective Disorders and Schizophrenia for School-Age Children – Present and Lifetime Version (K-SADS-PL)

The K-SADS-PL is a semi-structured psychiatric diagnostic interview that assesses present and lifetime conditions. K-SADS interviews was used in this study as the clinician-rated diagnostic criteria for Major Depressive Disorder and other psychiatric conditions.

- Clinical Global Impression (CGI)
- Children Depression Rating Scale (CDRS-R)

- Children’s Global Assessment Scale (CGAS)

Table 3.3 Self- and clinician-base instruments for phenotypic assessments for adolescents and primary caregivers From (Kieling et al., 2021).

Domain	Instrument
Adolescents	
Overall psychopathology	DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure, Child (CCSM-C) (24, 25)
Depression	Mood and Feelings Questionnaire—Child (MFQ-C) (26, 27)
Anhedonia	Snaith-Hamilton Pleasure Scale (SHAPS) (17, 28)
Irritability	Affective Reactivity Index—Child (ARI-C) (29, 30)
Suicidality	Columbia-Suicide Severity Rating Scale (C-SSRS) (31)
Anxiety	Spence Children's Anxiety Scale (SCAS-C) (32, 33)
Insomnia	Insomnia Severity Index (ISI) (34, 35)
Reflexive functioning	Reflective Functioning Questionnaire for Youth (RFQY) (36, 37)
Resilience	Adapted Resilience Scale (ARS)* (38, 39)
Positive attributes	Youth Strengths Inventory—Adolescent (YSI-A) (40, 41)
Parental bonding (separate measures for mother and father)	Parental Bonding Instrument (PBI) (42)
Maltreatment/trauma history	Child Trauma Questionnaire (CTQ) (43, 44)
Recent life events	Life Events Questionnaire (LEQ)* (45)
Physical activity	Patient-Centered Assessment and Counseling for Exercise Plus Nutrition* (PACE+) (46)
Primary caregivers	
Overall psychopathology	DSM-5 Self-Rated Level 1 Cross-Cutting Symptom Measure, Parent (CCSM-P) (24)
Depression	Mood and Feelings Questionnaire—Parent (MFQ-P) (26, 27)
Irritability	Affective Reactivity Index—Parent (ARI-P) (29, 30)
Anxiety	Spence Children's Anxiety Scale—Parent (SCAS-P) (32, 33)
Positive attributes	Youth Strengths Inventory—Parent (YSI-P) (40, 41)
Socioeconomic status	Brazil socioeconomic classification index (ABEP) (47)
Caregiver's depression	Mood and Feelings Questionnaire—Adult (MFQ-A) (26, 27)
Combined information (adolescent + caregiver)	
Depression	Children's Depression Rating Scale Revised (CDRS-R) (48, 49)
Clinical global impression	Clinical Global Impression (CGI) (50)
Global functioning	Children's Global Assessment Scale (CGAS) (51, 52)

3.1.5 IDEA RiSCo recruitment – Flowchart and details

The numbers of adolescents for each group to be recruited was defined *a priori* by the IDEA team, and the goal of the IDEA project was to recruit a final cohort of 150 adolescents represented by:

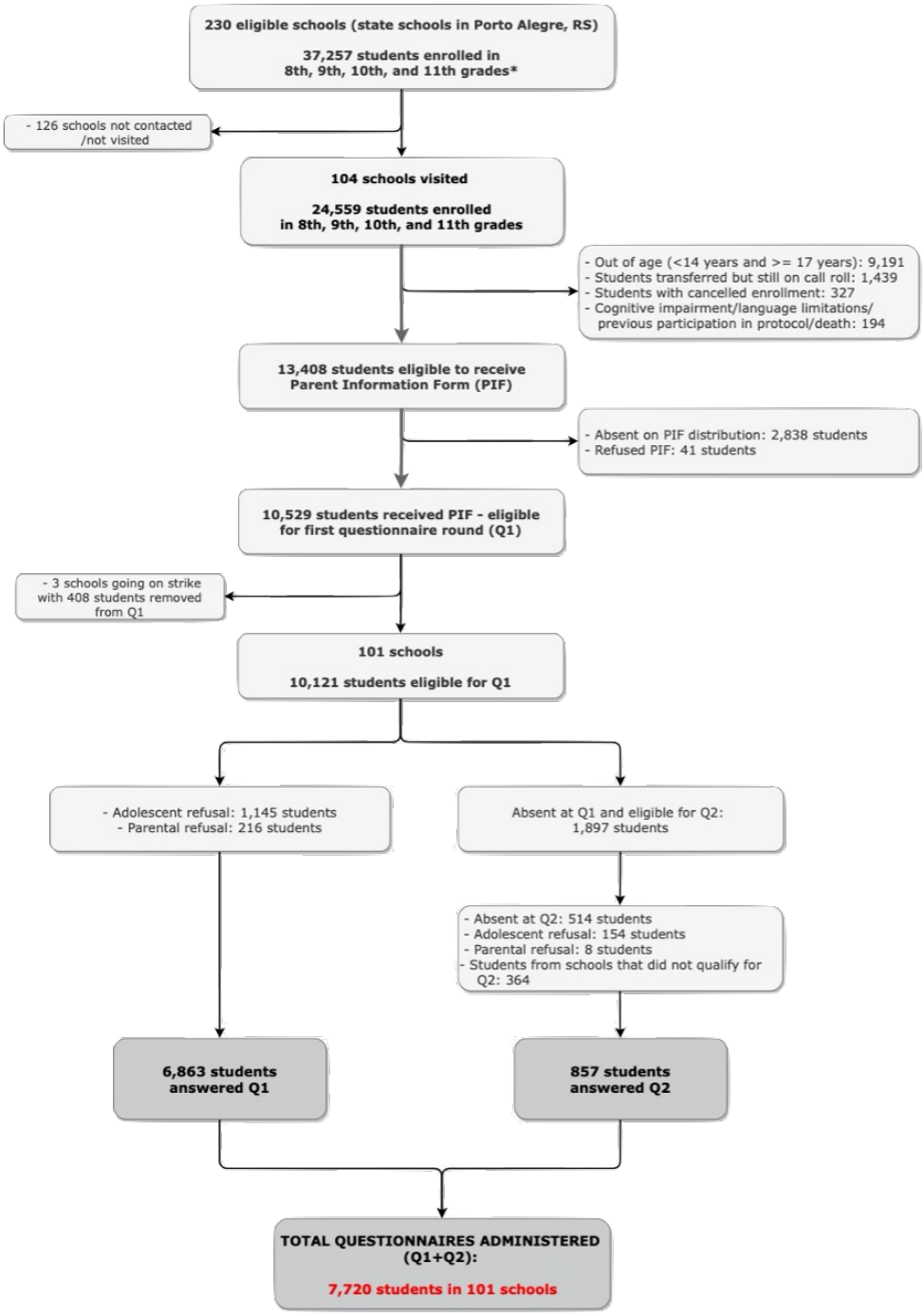
A. 50 High Risk Adolescents (25 females and 25 males);
B. 50 Low Risk Adolescents (25 females and 25 males);
C. 50 High Risk and MDD Adolescents (25 females and 25 males).

To reach this goal, the aforementioned recruitment process was done and in the following paragraphs the detailed flow chart and the numbers will be described to give a clear idea of the complexity of the recruitment.

In the city of Porto Alegre (Brazil) during the 2018, 104 public state schools agreed to participate to the IDEA project recruitment, for a total of 24,559 students in grades 8 to 11. Of this initial population of 24,559 students, 13,408 students were eligible for screening, whereas 11,151 were excluded because 9,191 were out of age (<14 years or ≥17 years); 1,439 were transferred to another state school; 327 cancelled enrollment; and 194 were excluded for cognitive impairment, language barriers, previous participation in the protocol at another school, or death.

On the day of the parent information form (PIF) distribution, the PIF was distributed to 10,529 students who became eligible to complete the screening; however, only 6,863 students completed the first questionnaire round (Q1). On the other hand, Q2 was administered to 857 students. Considering Q1 and Q2 administration, a total of 7,720 students completed screening questionnaires in 101 schools. The detailed flowchart is reported in figure 3.1, showing the exclusion criteria for each step.

Figure 3.1. Flowchart of school and student inclusion. From (Kieling et al., 2021)



Out of the 7,720 adolescents, 5,954 of them did not meet the inclusion criteria of right-handedness, no missing answers in the PHQ-A and IDEA-RS questionnaires, or classification into the LR, HR, or MDD groups. Thus, 1,766 were eligible for further assessment: 369 were classified as LR, 389 as HR, and 1,008 as MDD.

Parents or guardians were contacted over the phone and invited to accompany the adolescent to the Hospital de Clínicas de Porto Alegre (HCPA). Contact with 21, 81, and 506 participants in the LR, HR, and MDD groups respectively was not attempted because the target sample was met before they were called.

In the LR group 348 were contacted, among them 78 adolescents were scheduled for clinical evaluation and 64 of them underwent clinical evaluation. In the HR group, 308 were contacted, 97 adolescents were scheduled for clinical evaluation and 63 of them underwent clinical evaluation. In the MDD group, 502 were contacted, 166 adolescents were scheduled for clinical assessment and 133 of them underwent clinical evaluation.

3.1.6 Power Analysis

For the microarray technique, previous published data on transcriptomic experiments conducted in individuals characterized for stressful experiences (Lopizzo et al., 2017) or for depression development upon treatment with IFN-α (Hepgul et al., 2016) showed that a sample of 20 subjects per group is sufficient to identify changes in gene expression with a FDR corrected p-value <0.05 and a fold-change differences greater than 20%.

For the RNA Sequencing analysis, by using the ssizeRNA package, we determined that a sample size of 40 per group has a power of 92% for detecting significantly different

expressed genes, with FC of 2, FDR=0.05 and considering 15000 as the number of detected genes and 0.1 as the dispersion parameter for each gene (Bi & Liu, 2016).

3.2 Blood collection

Peripheral venous blood samples were collected in 2.5 mL PAXgene Blood RNA tubes (PreAnalitix, Qiagen / BD Company) in the morning, after an overnight fast. The PAXgene tubes contains an additive that stabilizes the *in vivo* gene transcription profile by reducing RNA degradation and minimizing gene induction. PAXgene tubes were collected as the last tubes of the phlebotomy procedure to prevent a possible backflow. The donor's arm was placed in a downward position after a butterfly needle was set for the withdrawal. The tube was hold in vertical position, below the donor's arm during blood collection and the tourniquet was released as soon as the blood started to flow into tube. After making sure that the blood stopped to flow into the PAXgene and the tube additive did not touch the stopper or the end of the needle during venepuncture, the tube was removed from the holder. Subsequently, the tube was gently inverted for 10 times and kept at room temperature for 2 hours, then frozen at -20°C for 24 hours and then moved to a –80°C freezer until their processing for nucleic acid extraction.

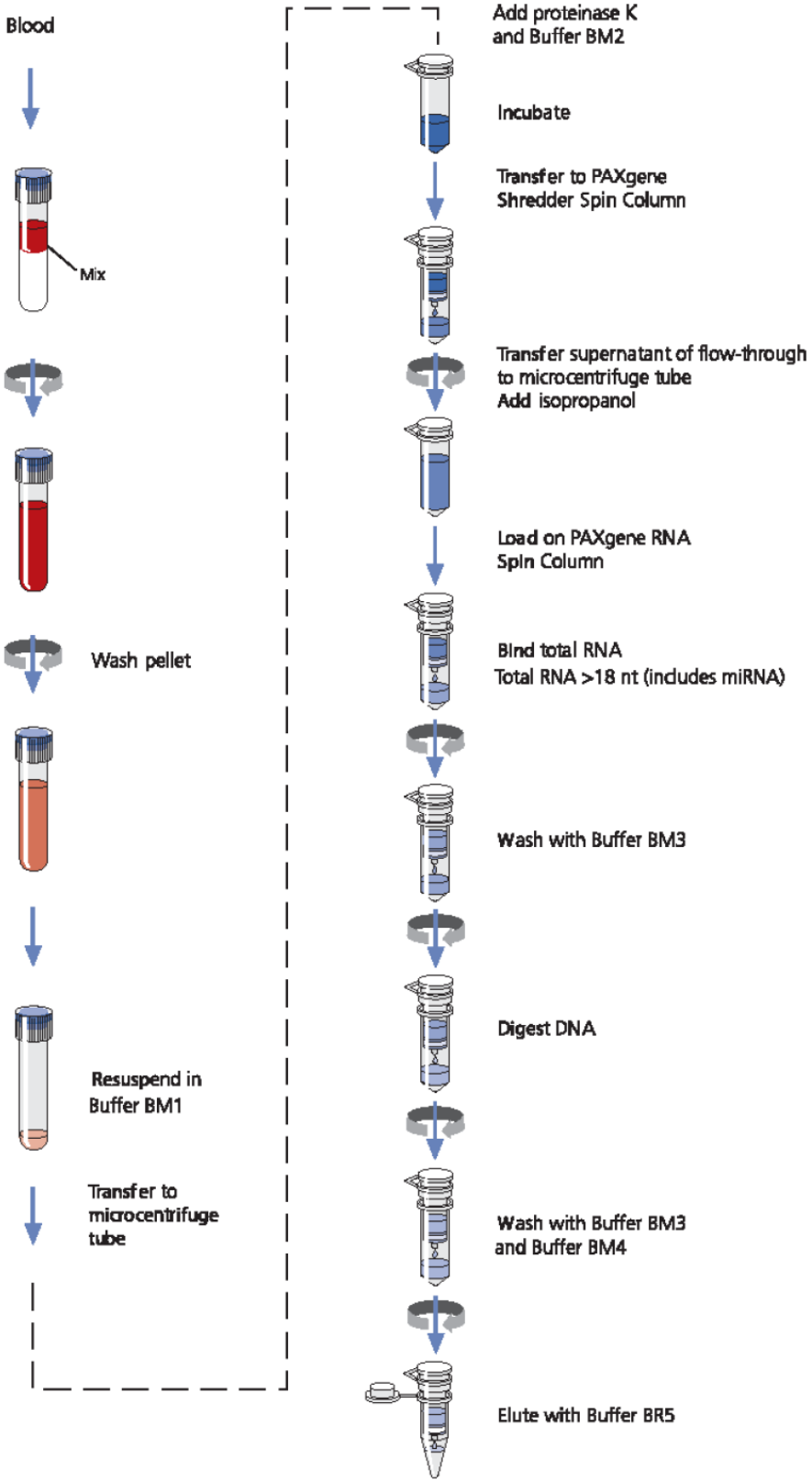
PAXgene tubes were shipped in dry ice and stored at – 80°C upon arrival.

3.3 RNA extraction from blood samples

Nucleic acid purification from PAXgene tubes was performed by using PAXgene Blood miRNA kit (Qiagen, Hilden, Germany; Cat No./ID: 763134) following an optimised protocol based on manufacturer's instruction.

Total RNA >18 nucleotides, including miRNAs, were purified from the stabilized blood samples using the PAXgene silica-membrane technology. PAXgene Blood RNA Tube was first centrifuged for 15 minutes at 3000 g to pellet the sample, which was then washed with RNAse-free water, recentrifuged as previously described and then resuspended in Buffer BM1. From this point, the RNA extraction followed the manufacturer's instruction (see figure 3.2 for a visual representation), which included also a 15-minute incubation with DNase enzyme, to digest DNA residues. As the last step, the sample was centrifuged through PAXgene RNA spin columns, where total RNA >18 nucleotides (including miRNA) bound to the PAXgene silica-membrane and were then eluted with BR5 buffer. Differently from the manufacturer's instruction, the eluted RNA was placed again in the silica-membrane and re-centrifuged, to increase the final yields. The extracted RNA was then immediately chilled on ice for subsequent quality control assessment or stored at -80°C for subsequent analysis.

Figure 3.2. RNA extraction procedure from PAXgene tube (PAXgene Blood miRNA Kit Handbook).



3.4 Quality control analysis

3.4.1 Nanodrop

RNA quantity and quality were assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA).

NanoDrop 2000 has a patented sample retention technology employing surface tension to hold the sample in place between two optical fibres. In this study, 1 µL of sample was pipetted onto the measurement pedestal; the optic fibre cable is embedded within this pedestal and a second optic cable is brought into contact with the liquid sample causing the liquid to bridge the gap between the two fibres. A pulsed xenon flash lamp provides the light source, and a spectrometer analyses the light passing through the sample. The nucleic acid concentration is measured by using a modified Beer-Lambert equation, consisting in using a factor with units of ng-cm/microliter. Here the equation:

$$\mathbf{c = (A * \varepsilon)/b}$$

c = the nucleic acid concentration in ng/microliter
A = the absorbance in AU
ε = the wavelength-dependent extinction coefficient in ng-cm/microliter
b= the pathlength in cm

The concentration is based on absorbance at 260 nm and the default or unit defined extinction coefficient.

Beside nucleic acid concentration, the following parameters are measured to provide a clear view of the sample quality:

- A260: absorbance at 260 nm normalized to a 10 mm pathlength;
- A280: absorbance at 280 nm normalized to a 10 mm pathlength;

- 260/280 ratio: used to assess the purity of RNA. A ratio of ~ 2.0 is accepted as pure. If this ratio is lower, it indicates the presence of protein, phenol or other contaminants that absorb near 280nm;
- 260/230 ratio: used to assess RNA purity as well. A ratio of 1.8 – 2.2 is considered as pure RNA; if it is appreciably lower, this may indicate the presence of copurified contaminants.

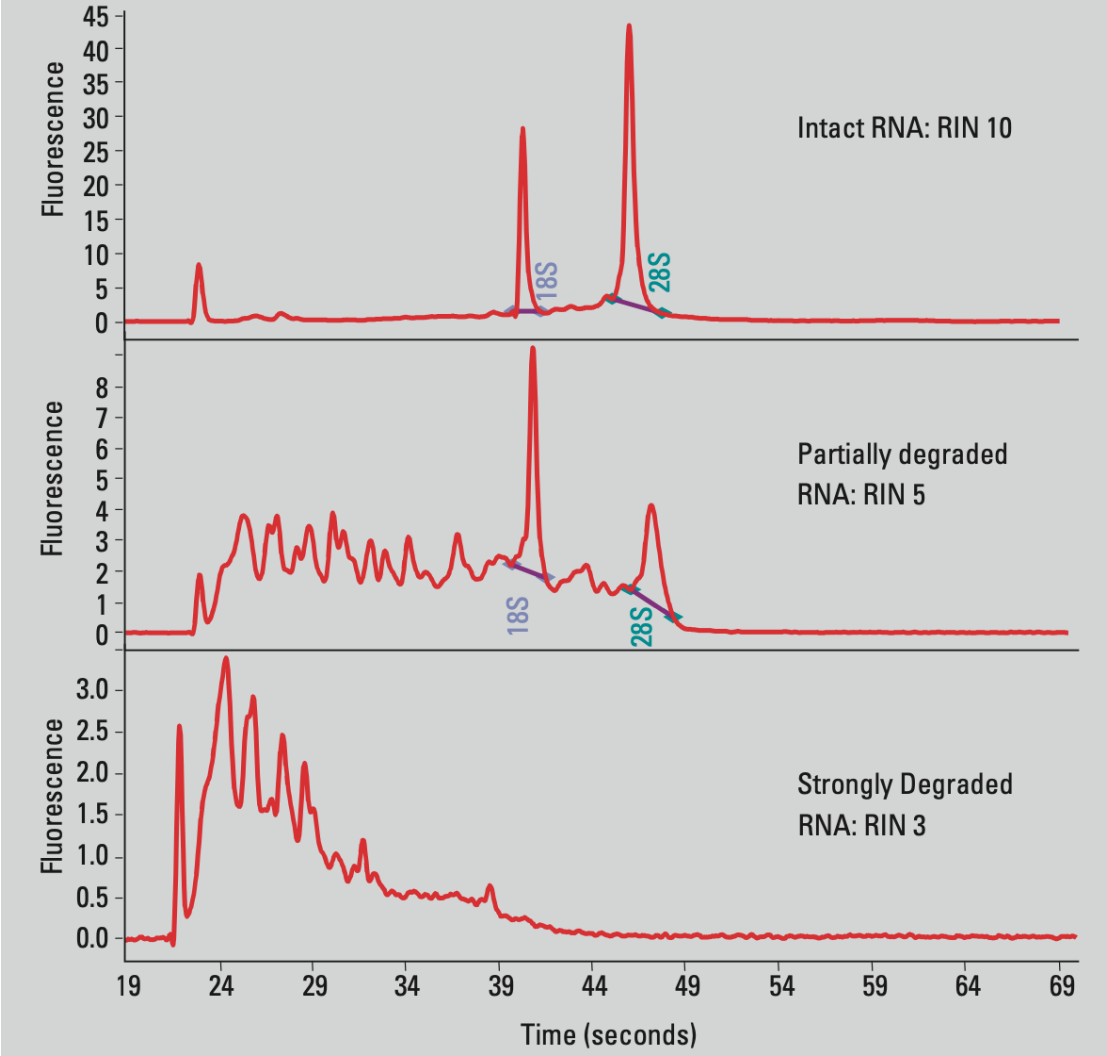
3.4.2 Agilent 2100 Bioanalyzer

Further quality control assessments were performed by using Agilent 2100 Bioanalyzer (Agilent, Santa Clara, United States) with RNA 6000 Nano Kit.

Using electrophoretic separation on microfabricated chips, RNA samples are separated and subsequently detected via laser induced fluorescence detection. The Bioanalyzer software generates an electropherogram and gel-like image and displays results such as sample concentration, the ribosomal ratio, and the RNA integrity number (RIN). The electropherogram provides a detailed visual assessment of the quality of an RNA sample.

The RIN is a software algorithm that indicates the RNA intactness by evaluation of the ribosomal ratio (18S and 28S) and it is based on a numbering system from 1 to 10, with 1 indicating a degraded profile and 10 the most intact (https://µL.agilent.com/cs/library/applications/5989-1165EN.pdf). Figure 3.3 represents an example of an electropherogram with different RIN values.

Figure 3.3. RNA integrity number tested on samples of varying levels of intactness. The RIN software algorithm was able to accurately classify the samples. From https://www.agilent.com/cs/library/applications/5989-1165EN.pdf



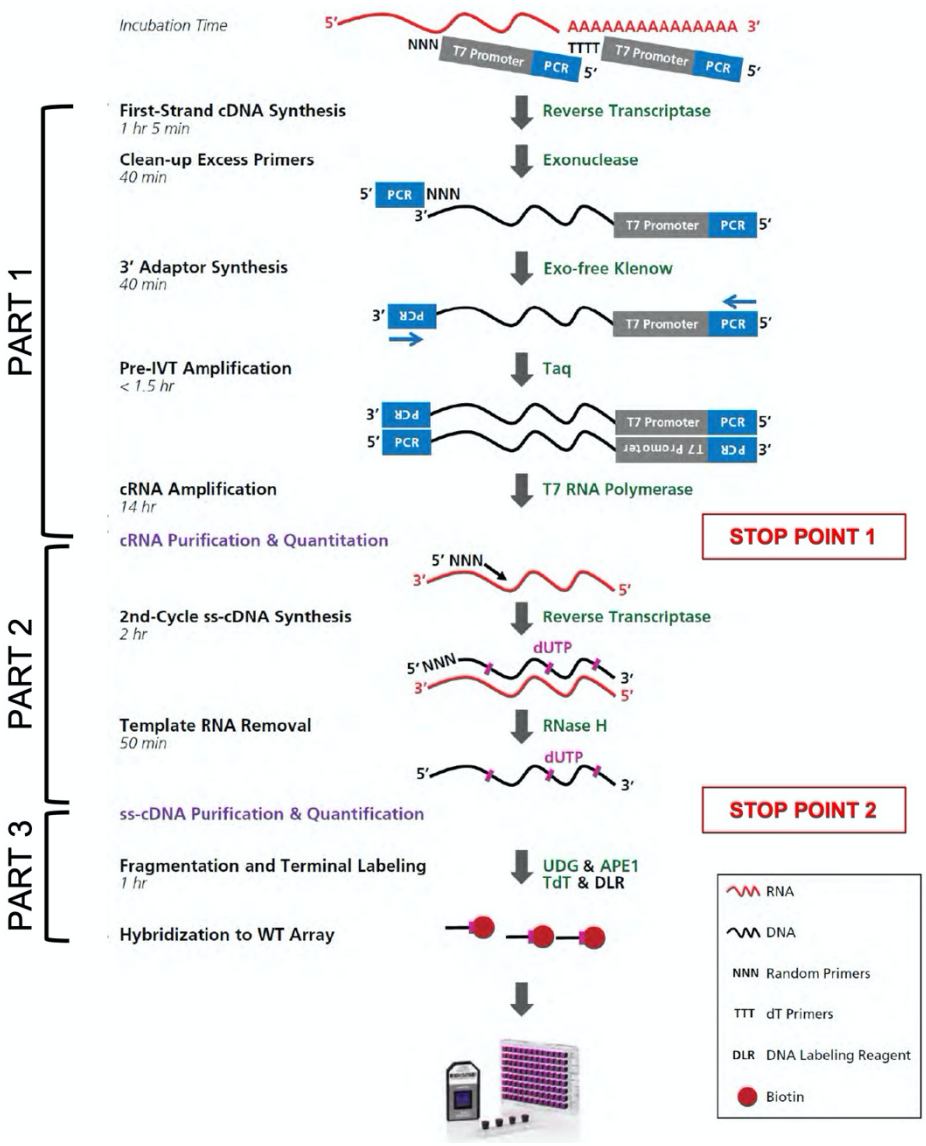
3.5 Genome-wide gene expression analysis on blood – Gene Atlas Affymetrix

3.5.1 Affymetrix pre-processing

Genome-wide gene expression analysis was performed starting from 10 ng of total RNA to prepare hybridized ready target for the subsequent labelling and fluorescent analysis.

The GeneChip WT Pico Reagent Kit (Cat. Number 902623, Thermo Fisher Scientific) allowed to obtain hybridized ready target from picogram amount of RNA, by following the manufacturer's instructions (schematized in figure 3.4).

Figure 3.4. GeneChip WT Pico Reagent Kit amplification and labeling process (GeneChip WT Pico Reagent Kit User Guide).



Firstly, Poly-A RNA controls were included in all the samples to be hybridized to GeneChip arrays. To include the premixed controls from the Poly-A RNA Control Strok, appropriate dilutions were prepared by following the scheme reported in table 3.4.

Table 3.4. Poly-A Controls stock dilutions

Total RNA Input Amount	1st Dilution	2nd Dilution	3rd Dilution	4th Dilution	Volume 4th Dilution to add to Total RNA
10 ng	1:50	1:100	1:100	1:10	2 µL

To prepare Poly-A RNA dilution for 10 ng of RNA, the following dilutions were prepared:

1. 1st dilution: add 2 µL of Poly-A Control Stock to 98 µL of nuclease-free water (1:50)
2. 2nd dilution: added 2 µL of 1st dilution to 198 µL of nuclease-free water (1:100)
3. 3rd dilution: added 2 µL of 2nd dilution to 198 µL of nuclease-free water (1:100)
4. 4th dilution: added 20 µL of third dilution to 180 µL of nuclease free water (1:10)
5. Added 2 µL of the 4th dilution to 10 ng of total RNA

Each dilution was mixed by vortexing and spinned down before being used for the subsequent dilution; fresh-dilution of Poly-A RNA Controls were prepared every day.

Total RNA extracted from PAXgene blood was diluted to obtain 10 ng of RNA in a total final volume of 3 µL by using the serial dilution technique. 2 µL of Poly-A Controls' 4th dilution were added to the RNA sample to obtain the final sample ready to be used for the following reactions.

As reported also in figure 3.4, the entire process before hybridization of the array strip was divided into three parts, each of them ending with a safe stop- point following the manufacturer's instruction. These safe stop -points were followed in these experiments.

Moreover, the 150 blood samples were differently randomized for each of the three phases, by using an online randomizing tool (https://www.random.org/lists). The randomized groups are reported in table 3.5.

Table 3.5. Randomization of the 150 samples accordingly to risk groups A, B and C for the pre-processing (Part 1)

1. BR0015PAX C	GROUP 1	49. BR0051PAX B	GROUP 4	97. BR0038PAX C	GROUP 7	145. BR0123PAX C	9
2. BR0045PAX C		50. BR0002PAX C		98. BR0082PAX B		146. BR0153PAX B	
3. BR0076PAX B		51. BR0020PAX A		99. BR0142PAX B		147. BR0164PAX B	
4. BR0137PAX B		52. BR0077PAX A		100. BR0089PAX A		148. BR0165PAX C	
5. BR0040PAX C		53. BR0058PAX C		101. BR0088PAX A		149. BR0170PAX C	
6. BR0094PAX C		54. BR0157PAX B		102. BR0080PAX B		150. BR0134PAX C	
7. BR0083PAX C		55. BR0120PAX B		103. BR0114PAX B			
8. BR0144PAX B		56. BR0097PAX A		104. BR0050PAX B			
9. BR0091PAX C		57. BR0128PAX B		105. BR0064PAX C			
10. BR0101PAX A		58. BR0085PAX B		106. BR0093PAX A	GROUP 8		
11. BR0005PAX A		59. BR0047PAX A		107. BR0118PAX A			
12. BR0018PAX C		60. BR0036PAX A		108. BR0130PAX A			
13. BR0056PAX C		61. BR0081PAX B		109. BR0141PAX B			
14. BR0033PAX A		62. BR0037PAX A		110. BR0146PAX A			
15. BR0099PAX B		63. BR0074PAX A		111. BR0116PAX A			
16. BR0043PAX C		64. BR0104PAX A		112. BR0158PAX B			
17. BR0087PAX B	GROUP 2	65. BR0071PAX A	GROUP 5	113. BR0169PAX C			
18. BR0121PAX A		66. BR0109PAX A		114. BR0172PAX C			
19. BR0060PAX A		67. BR0066PAX A		115. BR0098PAX A			
20. BR0107PAX A		68. BR0113PAX B		116. BR0145PAX B			
21. BR0131PAX B		69. BR0024PAX C		117. BR0125PAX A			
22. BR0075PAX A		70. BR0073PAX C		118. BR0129PAX A			
23. BR0053PAX B		71. BR0140PAX A		119. BR0102PAX A			
24. BR0028PAX C		72. BR0059PAX B		120. BR0156PAX C			
25. BR0078PAX B		73. BR0057PAX C		121. BR0162PAX B			
26. BR0027PAX C		74. BR0055PAX C		122. BR0168PAX B			
27. BR0151PAX B		75. BR0054PAX B		123. BR0139PAX B			
28. BR0032PAX C		76. BR0112PAX A		124. BR0124PAX C			
29. BR0048PAX A		77. BR0111PAX A		125. BR0148PAX B			
30. BR0086PAX A		78. BR0103PAX C		126. BR0160PAX B			
31. BR0152PAX B		79. BR0052PAX B		127. BR0135PAX B			
32. BR0044PAX C		80. BR0115PAX B		128. BR0171PAX C			
33. BR0095PAX A	GROUP 3	81. BR0070PAX A	GROUP 6	129. BR0154PAX A	GROUP 9		
34. BR0067PAX B		82. BR0049PAX A		130. BR0133PAX B			
35. BR0039PAX C		83. BR0019PAX C		131. BR0150PAX A			
36. BR0035PAX B		84. BR0100PAX B		132. BR0149PAX A			
37. BR0013PAX B		85. BR0108PAX A		133. BR0127PAX C			
38. BR0096PAX A		86. BR0106PAX B		134. BR0167PAX C			
39. BR0069PAX A		87. BR0084PAX B		135. BR0138PAX A			
40. BR0001PAX C		88. BR0022PAX C		136. BR0163PAX C			
41. BR0029PAX C		89. BR0046PAX C		137. BR0147PAX B			
42. BR0065PAX C		90. BR0143PAX A		138. BR0173PAX C			
43. BR0030PAX C		91. BR0092PAX A		139. BR0132PAX C			
44. BR0079PAX B		92. BR0021PAX C		140. BR0119PAX A			
45. BR0006PAX C		93. BR0009PAX C		141. BR0090PAX C			
46. BR0072PAX A		94. BR0061PAX C		142. BR0161PAX B			
47. BR0122PAX C		95. BR0110PAX A		143. BR0155PAX A			
48. BR0105PAX B		96. BR0117PAX B		144. BR0136PAX B			

PART 1. The first-strand cDNA was synthesized by mixing 5 µL of total RNA, 4 µL of WT Pico First-Strand buffer and 1 µL of WT Pico First-Strand enzyme. This reaction mix was incubated for 5 minutes at 25°C, 60 minutes at 42°C and then for at least 2 minutes at 4°C. After being centrifuged and chilled on ice for 2 minutes to cool the plastic, 2 µL of WT Pico Cleanup Reagent were added to each 10 µL of cDNA sample while on ice. After adding every reagent, the sample was always mixed thoroughly (no vortex) and centrifuged briefly to remove air bubbles and collect the reaction at the bottom of the tube. The reaction mix was incubated for 30 minutes at 37°C, 10 minutes at 80°C and for at least 2 minutes at 4°C. Then, it was centrifuged briefly to collect the first-strand cDNA at the bottom and placed on ice for 2 minutes to cool the plastic.

Subsequently, 3' Adaptor was added to the single-stranded cDNA, which acted as a template for a double-stranded cDNA synthesis in the pre-*in vitro* amplification reaction. WT pico 3' Adaptor buffer (7 µL) and enzyme (1 µL) were added to the First-Strand cDNA sample and incubated for 15 minutes at 15°C, 15 minutes at 35°C, 10 minutes at 70°C and then for at least 2 minutes at 2°C. This reaction was performed on a thermal cycler with the lid kept off. After the incubation, the samples were collected at the bottom of the tube and cool on ice.

Then, single-stranded cDNA was converted to double-stranded cDNA, which acted as a template for the *in vitro* transcription. This reaction used TaqDNA polymerase and Adaptor-specific primers to synthesize and pre-amplify double-stranded cDNA. WT pico PCR Buffer (29 µL) and enzyme (1 µL) were added to the previous mix and incubated at 2 minutes for 95°C, followed by 6 cycles at 30 second at 94°C and 5 minutes at 70°C, and then for at least 2 minutes at 4°C.

The following reaction was the *in vitro* Transcription (IVT), in which the double-stranded cDNA template was used for synthesizing and amplifying the anti-sense RNA (complimentary or cRNA) using T7 RNA polymerase. While at room temperature, 24 µL of WT Pico IVT buffer and 1 µL of enzyme were added and the mix incubated at 40°C for 15 hours (overnight reaction).

After the 15 hours of IVT, the cRNA product was cleared by enzymes, salts, inorganic phosphates, and unincorporated nucleotides. The purification was performed by using magnetic purification beads, able to bind cRNA. The process consisted in several washing steps with ethanol 80% and final dilution with nuclease-free water.

At this point, the purified cRNA was immediately chilled on ice for quality control assessment by using Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To proceed to the next step (2^{nd}-cycle single-stranded cDNA) the lowest cRNA concentration required was 833 ng/µL. Purified cRNA samples with a concentration higher than 833 ng/µL were stored at -80°C (first STOP POINT); on the other hand, samples with lower concentration required to be pre-processed starting from fresh 10 ng total RNA.

All 150 samples were pre-processed for part 1 before proceeding with part 2. Before proceeding with pre-processing Part 2, samples were randomized again. The randomized groups are reported in table 3.6.

Table 3.6. Randomization of the 150 samples accordingly to risk groups A, B and C for the pre-processing (Part 2)

1. BR0086PAX A	GROUP 1	57. BR0015PAX C	GROUP 4	113.BR0145PAX B	6
2. BR0094PAX C		58. BR0113PAX B		114. BR0172PAX C	
3. BR0105PAX B		59. BR0051PAX B		115. BR0153PAX B	
4. BR0052PAX B		60. BR0055PAX C		116. BR0124PAX C	
5. BR0061PAX C		61. BR0082PAX B		117. BR0141PAX B	
6. BR0079PAX B		62. BR0114PAX B		118. BR0171PAX C	
7. BR0040PAX C		63. BR0143PAX A		119. BR0116PAX A	
8. BR0096PAX A		64. BR0045PAX C		120. BR0118PAX A	
9. BR0104PAX A	GROUP 2	65. BR0056PAX C		121. BR0139PAX B	
10. BR0024PAX C		66. BR0036PAX A		122. BR0155PAX A	
11. BR0157PAX B		67. BR0152PAX B		123. BR0147PAX B	
12. BR0120PAX B		68. BR0095PAX A		124. BR0132PAX C	
13. BR0142PAX B		69. BR0109PAX A		125. BR0165PAX C	
14. BR0110PAX A		70. BR0047PAX A		126. BR0090PAX C	
15. BR0001PAX C		71. BR0030PAX C		127. BR0164PAX B	
16. BR0108PAX A		72. BR0081PAX B		128. BR0149PAX A	
17. BR0046PAX C		73. BR0140PAX A		129. BR0160PAX B	GROUP 7
18. BR0013PAX B		74. BR0101PAX A		130. BR0102PAX A	
19. BR0121PAX A		75. BR0065PAX C		131. BR0169PAX C	
20. BR0022PAX C		76. BR0131PAX B		132. BR0134PAX C	
21. BR0002PAX C		77. BR0028PAX C		133. BR0130PAX A	
22. BR0144PAX B		78. BR0074PAX A		134. BR0168PAX B	
23. BR0075PAX A		79. BR0137PAX B		135. BR0119PAX A	
24. BR0053PAX B		80. BR0070PAX A		136. BR0133PAX B	
25. BR0112PAX A	GROUP 3	81. BR0064PAX C	GROUP 5	137. BR0125PAX A	
26. BR0019PAX C		82. BR0089PAX A		138. BR0167PAX C	
27. BR0092PAX A		83. BR0009PAX C		139. BR0138PAX A	
28. BR0100PAX B		84. BR0049PAX A		140. BR0154PAX A	
29. BR0033PAX A		85. BR0073PAX C		141. BR0156PAX C	
30. BR0072PAX A		86. BR0020PAX A		142. BR0098PAX A	
31. BR0103PAX C		87. BR0067PAX B		143. BR0123PAX C	
32. BR0085PAX B		88. BR0122PAX C		144. BR0158PAX B	
33. BR0088PAX A		89. BR0080PAX B		145. BR0127PAX C	
34. BR0021PAX C		90. BR0006PAX C		146. BR0150PAX A	
35. BR0106PAX B		91. BR0066PAX A		147. BR0161PAX B	
36. BR0037PAX A		92. BR0115PAX B		148. BR0136PAX B	
37. BR0087PAX B		93. BR0039PAX C		149. BR0093PAX A	
38. BR0069PAX A		94. BR0083PAX C		150. BR0163PAX C	
39. BR0097PAX A		95. BR0107PAX A			
40. BR0076PAX B		96. BR0005PAX A			
41. BR0029PAX C		97. BR0054PAX B			
42. BR0091PAX C		98. BR0032PAX C			
43. BR0035PAX B		99. BR0043PAX C			
44. BR0018PAX C		100. BR0099PAX B			
45. BR0044PAX C		101. BR0050PAX B			
46. BR0077PAX A		102. BR0038PAX C			
47. BR0151PAX B		103. BR0128PAX B			
48. BR0071PAX A		104. BR0048PAX A			
49. BR0117PAX B		105. BR0059PAX B	GROUP 6		
50. BR0027PAX C		106. BR0162PAX B			
51. BR0060PAX A		107. BR0129PAX A			
52. BR0058PAX C		108. BR0170PAX C			
53. BR0111PAX A		109. BR0148PAX B			
54. BR0084PAX B		110. BR0135PAX B			
55. BR0057PAX C		111. BR0146PAX A			
56. BR0078PAX B		112. BR0173PAX C			

PART 2. Sense-strand cDNA was synthetized by the reverse transcription of 20 µg of cRNA using 2nd-cycle primers. The sense-strand cDNA contained dUTP at a fixed ratio relative to dTTP. Firstly, while on ice, 20 µg of cRNA were diluted into 24 µL of nuclease-free water. Then, the 2nd-cycle ss-cDNA mix was prepared by adding 49 µL of WT Pico 2nd-cycle ss-cDNA primers, 8 µL of buffer and 1 µL of enzyme; the reaction mix was incubated for 10 minutes at 25°C, 90 minutes at 42°C, 10 minutes at 70°C and then for at least 2 minutes at 4°C.

The next step was the RNase H hydrolyzation of the cRNA template, to leave only the single-stranded cDNA. 4µL of RNase H were added to the 2nd-cycle ss-cDNA samples and incubated for 45 minutes at 37°C, 5 minutes at 95°C and then for at least 2 minutes at 4°C. After a brief centrifugation to collect the sample on the bottom of the tube, 11 µL of nuclease-free water were added to each hydrolysed 2nd-cycle ss-cDNA sample. After that, the sample faced the second purification with the magnetic beads. Once purified, the eluted ss-cDNA quality was assessed by using the Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA). To proceed to the next step, a minimum concentration of 120 ng/µL was required. After the Nanodrop assessment, samples were stored at -20°C (second STOP POINT).

All 150 samples were pre-processed for part 2 before proceeding for part 3. Before proceeding with pre-processing Part 3 and array strip hybridization, samples were randomized again. The randomized groups are reported in table 3.7.

Table 3.7. Randomization of the 150 samples accordingly to risk groups A, B and C for the array-strips (Part 3)

BR0005PAX A	ARRAY STRIP 1	BR0109PAX A	ARRAY STRIP 11	BR0169PAX C	ARRAY STRIP 21	BR0054PAX B	ARRAY STRIP 31
BR0043PAX C		BR0013PAX B		BR0037PAX A		BR0158PAX B	
BR0093PAX A		BR0122PAX C		BR0118PAX A		BR0094PAX C	
BR0100PAX B		BR0102PAX A		BR0030PAX C		BR0027PAX C	
BR0097PAX A	ARRAY STRIP 2	BR0040PAX C	ARRAY STRIP 12	BR0071PAX A	ARRAY STRIP 22	BR0070PAX A	ARRAY STRIP 32
BR0056PAX C		BR0128PAX B		BR0144PAX B		BR0079PAX B	
BR0171PAX C		BR0112PAX A		BR0154PAX A		BR0096PAX A	
BR0114PAX B		BR0006PAX C		BR0036PAX A		BR0082PAX B	
BR0172PAX C	ARRAY STRIP 3	BR0157PAX B	ARRAY STRIP 13	BR0098PAX A	ARRAY STRIP 23	BR0099PAX B	ARRAY STRIP 33
BR0086PAX A		BR0121PAX A		BR0045PAX C		BR0165PAX C	
BR0113PAX B		BR0021PAX C		BR0046PAX C		BR0138PAX A	
BR0048PAX A		BR0135PAX B		BR0081PAX B		BR0134PAX C	
BR0047PAX A	ARRAY STRIP 4	BR0137PAX B	ARRAY STRIP 14	BR0074PAX A	ARRAY STRIP 24	BR0173PAX C	ARRAY STRIP 34
BR0142PAX B		BR0069PAX A		BR0130PAX A		BR0163PAX C	
BR0089PAX A		BR0145PAX B		BR0067PAX B		BR0155PAX A	
BR0064PAX C		BR0057PAX C		BR0103PAX C		BR0127PAX C	
BR0083PAX C	ARRAY STRIP 5	BR0116PAX A	ARRAY STRIP 15	BR0052PAX B	ARRAY STRIP 25	BR0133PAX B	ARRAY STRIP 35
BR0059PAX B		BR0055PAX C		BR0001PAX C		BR0090PAX C	
BR0073PAX C		BR0032PAX C		BR0050PAX B		BR0150PAX A	
BR0111PAX A		BR0160PAX B		BR0038PAX C		BR0147PAX B	
BR0058PAX C	ARRAY STRIP 6	BR0140PAX A	ARRAY STRIP 16	BR0015PAX C	ARRAY STRIP 26	BR0149PAX A	ARRAY STRIP 36
BR0148PAX B		BR0018PAX C		BR0124PAX C		BR0132PAX C	
BR0044PAX C		BR0088PAX A		BR0125PAX A		BR0164PAX B	
BR0110PAX A		BR0061PAX C		BR0139PAX B		BR0167PAX C	
BR0156PAX C	ARRAY STRIP 7	BR0024PAX C	ARRAY STRIP 17	BR0066PAX A	ARRAY STRIP 27	BR0119PAX A	ARRAY STRIP 37
BR0108PAX A		BR0143PAX A		BR0084PAX B		BR0153PAX B	
BR0002PAX C		BR0151PAX B		BR0078PAX B		BR0161PAX B	
BR0072PAX A		BR0095PAX A		BR0022PAX C		BR0123PAX C	
BR0107PAX A	ARRAY STRIP 8	BR0141PAX B	ARRAY STRIP 18	BR0077PAX A	ARRAY STRIP 28	BR0170PAX C	ARRAY STRIP 38
BR0080PAX B		BR0033PAX A		BR0075PAX A		BR0136PAX B	
BR0152PAX B		BR0146PAX A		BR0053PAX B			
BR0092PAX A		BR0168PAX B		BR0019PAX C			
BR0101PAX A	ARRAY STRIP 9	BR0104PAX A	ARRAY STRIP 19	BR0105PAX B	ARRAY STRIP 29		
BR0117PAX B		BR0051PAX B		BR0106PAX B			
BR0085PAX B		BR0035PAX B		BR0129PAX A			
BR0029PAX C		BR0020PAX A		BR0060PAX A			
BR0049PAX A	ARRAY STRIP 10	BR0162PAX B	ARRAY STRIP 20	BR0065PAX C	ARRAY STRIP 30		
BR0087PAX B		BR0009PAX C		BR0131PAX B			
BR0028PAX C		BR0115PAX B		BR0091PAX C			
BR0076PAX B		BR0039PAX C		BR0120PAX B			

PART 3. The third and last part of the pre-processing consisted in the fragmentation and labelling of the single-stranded cDNA. The ss-cDNA was fragmented by uracil-DNA glycosylase (UDG) and apurinic/apyrimidinic endonuclease 1 (APE 1). The fragmented cDNA was then labelled by terminal deoxynycleotidyl transferase (TdT) using a DNA labelling reagent which was covalently linked to biotin. Firstly, 5.5 µg of ss-cDNA were prepared in a volume of 46 µL of nuclease-free water. Secondly, WT Pico Fragmentation and Label buffer (12 µL) and enzyme (2 µL) were added to the ss-cDNA and incubated for 1 hour at 37°C, 2 minutes at 93°C and for at least 2 minutes at 4°C. Once centrifuged briefly and placed on ice, the sample were ready for array hybridization.

3.5.2 Array Strip hybridization on the Gene Atlas System

HuGene 2.1 Array Strips were used in this phase. Before starting the hybridization, the array strips and 5X WT Hyb Add 1, 15X WT Hyb Add 4 and 2.5X WT Hyb Add 6 were allowed to equilibrate at room temperature; Control Oligonucleotide B2 (2nM) and 20X Eukaryotic Hybridization Controls were thawed at room temperature while on ice. Firstly, 20X Hybridization Controls were incubated for 5 minutes at 65°C; after that, the hybridization master mix was prepared in a nuclease-free tube while at room temperature by adding 5X wt Hyb Add 1 (30 µL for one array), control oligonucleotide B2 (1.5 µL), 20X hybridization controls (7.6 µL), 15X WT Add 4 (10 µL), mixed gently, and centrifuged to collect the mix at the bottom of the tube. After that, the hybridization cocktail for a single array was prepared by adding 49 µL of the hybridization master mix to 41 µL of fragmented and labelled ss-cDNA and 60µL of 2.5X WT hyb Add 6. The hybridization cocktail was vortexed, centrifuged and then

incubated for 5 minutes at 99°C and then for 5 minutes at 45°C. Before proceeding with the hybridization, the array strip was registered on the Gene Atlas platform following manufacturer's instructions.

Then, 120 µL of Hybridization cocktail were applied to the middle of the appropriate wells of a new clean hybridization tray and the array strip placed into the hybridization tray being careful to not scratch or damage the array surface as well as not make any air bubbles. At this point, the array strip was scanned onto the platform and then the Gene Atlas Hybridization Station temperature was set at 48°C; the array strip was carefully inserted, the station clamp closed, and the 20 hours' countdown started (hybridization phase).

After the hybridization, the array strip was moved to the Gene Atlas Fluidic Station for the wash and staining process. Once finished, the array strip was moved to the Imager for the last step, when the image was acquired and the CEL file produced for the bioinformatic analysis.

3.5.3 Quality control

The internal quality controls for gene expression monitor the assay data quality and are represented by signal value, hybridization controls, labelling controls, and internal control genes. The results of these controls are displayed by the QC report. If one of the parameters does not reach the pre-defined thresholds, it is shown in the summary of the QC status.

3.6 Bioinformatic analysis – Gene Atlas Affymetrix

3.6.1 Partek software

The Affymetrix CEL file were imported into the Partek Genomic Suite software and the analysis followed a specific pipeline. After importing the samples and giving attributes, the quality assessment and control were performed (QA/QC analysis).

The data were firstly explored with the Principal Components Analysis (PCA) to find similarities and differences between the samples. In the PCA scatter plot each point represents a chip (sample) and corresponds to a row on the top-level spreadsheet; points that are close together in the plot have similar intensity values across the probe sets on the whole chip, while points that are far apart in the plot are dissimilar. It is possible to configurate the colour and dimension of points of the scatter plot based on different variables, such as tissue, type, gender, and scan date. The PCA scatter plot allows to identify outliers or the so-called batch effects, which depends on differences in the intensity due to the scan date (an example is represented in figure 3.5). Moreover, it is possible to identify outlier by looking at the sample histogram (or Box and Whiskers Chart), where the intensity of the probes is graphed on the X-axis and the frequency of the probe intensity on the y-axis (an example is represented in figure 3.6 and 3.7).

Background correction was conducted using Robust Multi-strip Average (RMA) (Irizarry et al., 2003) to remove noise from auto fluorescence. After background correction, normalization was conducted using Quantiles normalization (Bolstad, Irizarry, Astrand, & Speed, 2003) to normalize the distribution of probe intensities among different microarray chips. Subsequently, a summarization step was

conducted using a linear median polish algorithm to integrate probe intensities to compute the expression levels for each gene transcript.

After the QA/QC, the differences among the three groups were assessed by using an analysis of variance (ANOVA). To quantify the relative contribution of each factor in the model towards explaining the variability of the data, it was necessary to consider the Sources of Variation plot. Lastly, gene lists of differently expressed gene among the different groups were creating from the ANOVA.

Differential gene expression across treatment was assessed by applying a p-value filter of $p < 0.05$ to the ANOVA results, a fold-change cut-off of $\pm |1.2|$ and a Benjamini-Hochberg adjusted false discovery rate (FDR) cut-off of 0.05 (q-value).

Figure 3.5. Partek Genomic Suite PCA for Scan Date

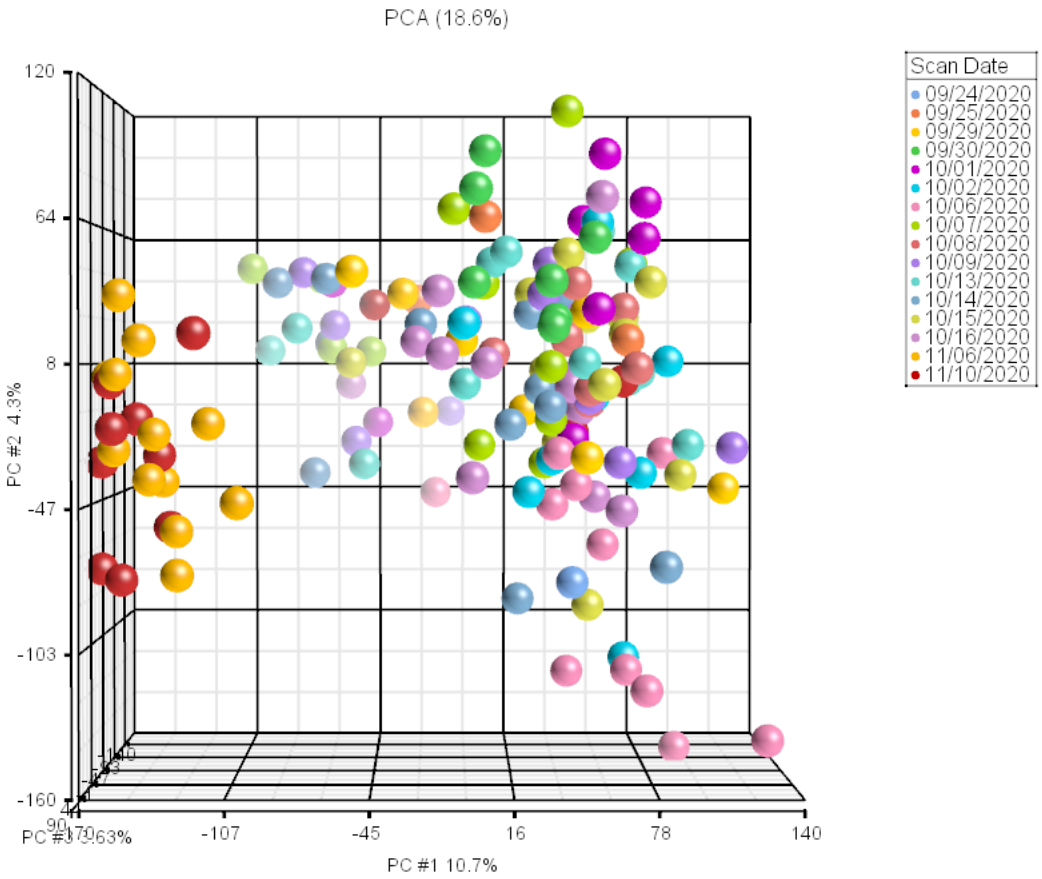


Figure 3.6. Partek Genomic Suite Histogram for Intensity (divided for Scan Date)

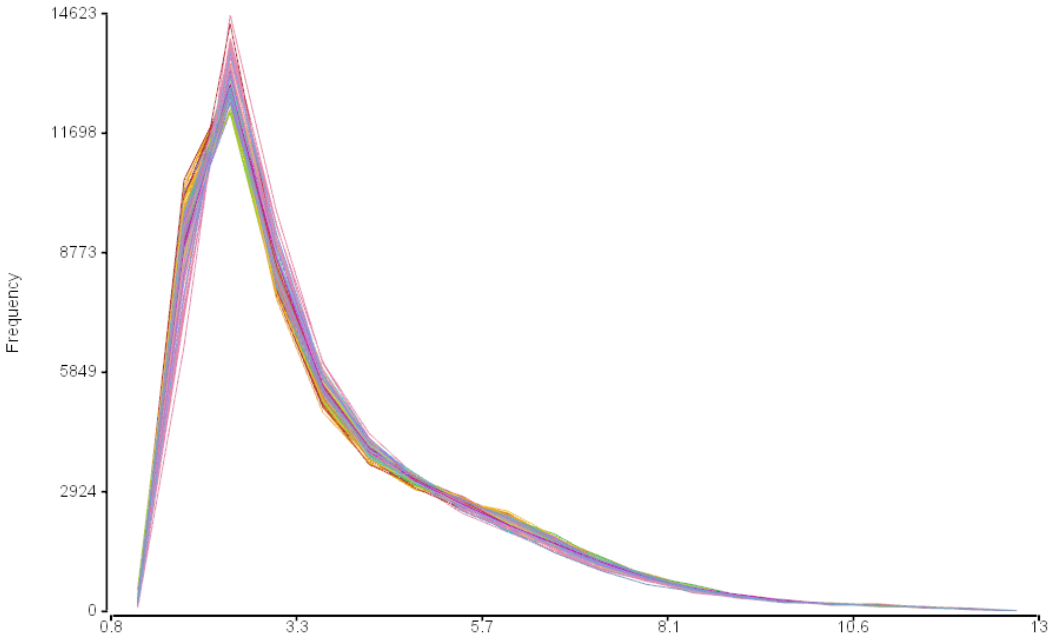
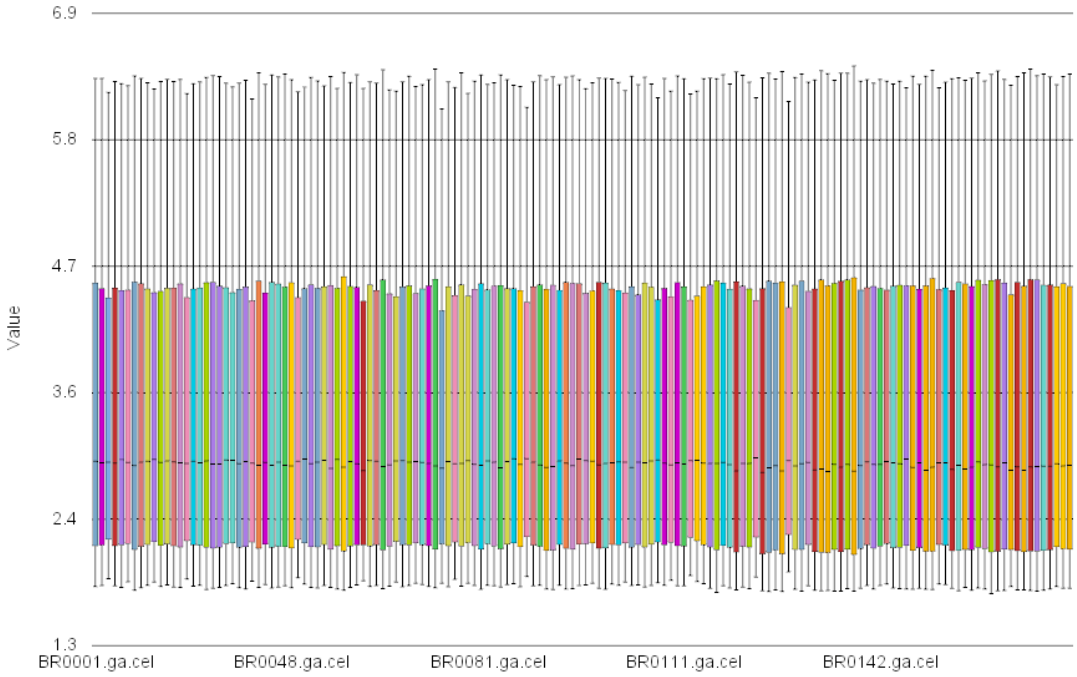


Figure 3.7. Partek Genomic Suite Box and Whiskers Chart (divided for Scan Date)



3.6.2 Pathways analysis

Genes differentially modulated across groups were then used to run a pathway analysis by using Ingenuity Pathway Analyses Software (Qiagen). Specifically, IPA software requires to upload lists of genes including columns of measurements about each gene, such as fold-changes and p-values. The software detects the pathway overlap of the significant dataset molecules, by using an algorithm that compares the up-regulated and the down-regulated genes in the uploaded data to the pattern expected for that specific pathway. Indeed, up-regulated genes might activate or inhibit the pathways, depending on the role of that genes which is thus accurately balanced and recognized by the software. The results of the pathways analysis are reported in the "Core Analysis" tab, which reported the lists of pathways significantly associated with the list of genes differently expressed, together with their fold-change and p-values. The statistical significance of the overlap of the uploaded datasets of DEGs is calculated by using the Fisher's Exact Test. The calculation of the p-value for the identification of the pathways differentially expressed depends on several factor, specifically : 1) the number of molecules associated with a given pathways; 2) the number of eligible analysis-ready molecules from the database that participate in the annotation and are also in the uploaded reference set of genes; 3) the total number of molecules known to be associated with that annotation and that are in the reference set of DEGs uploaded; 4) the total number of molecules in the reference set; 5) the total number of analysis-ready molecules that did not match that annotation. However, the IPA software does not apply a Bonferroni correction or any other correction because of a possible overcorrection of the results, leading thus to a high false negative rate accordingly to the IPA user manual. Moreover, as reported on the

user manual, it is recommended to do not use multiple testing correction in analysing canonical pathways, and this advice was followed in this doctoral thesis.

In the following paragraph, each pathway will be reported with its p-value and its z-score. Conceptually, the z-score is a statistical measure of how closely the actual expression pattern of the DEGs in the uploaded datasets compare to the pattern that is expected based on the literature for each specific pathway identified. Z-scores > 2 or < -2 are considered significant; specifically, z-score > 2 means a predicted activation of that pathway (an up-regulation), whereas a z-score < -2 means a predicted inactivation (a down-regulation) (Kramer, Green, Pollard, & Tugendreich, 2014).

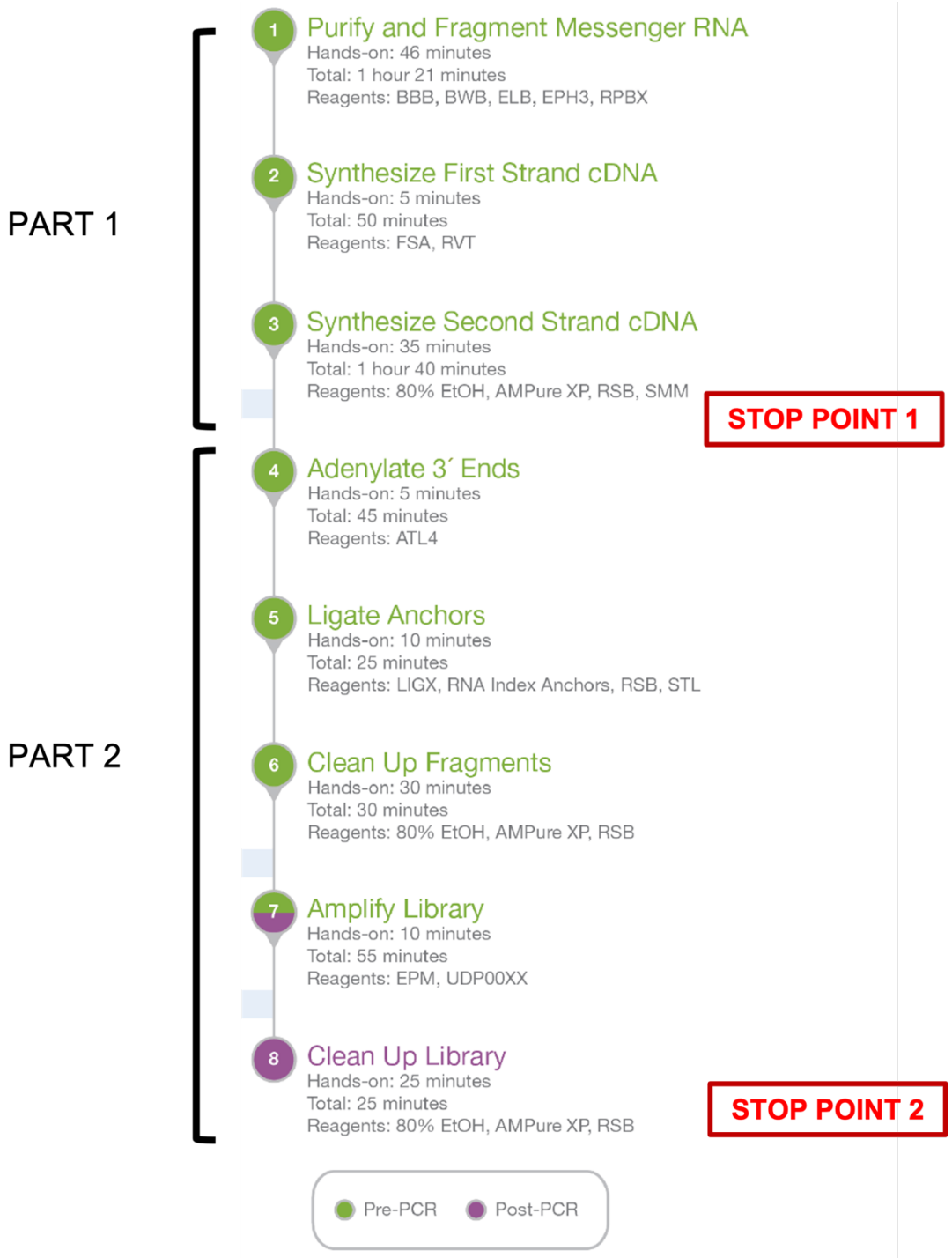
3.7 RNA Sequencing on blood – NextSeq 550

3.7.1 Dual-indexed libraries preparation

The RNA sequencing on the 150 blood samples was performed starting from 150 ng of total RNA previously extracted from PAX-Gene tubes by using the Illumina Stranded mRNA Prep Ligation Kit, which converts the messenger (mRNA) in total RNA into dual-indexes libraries. Briefly, Oligo(dT) magnetic beads purify and capture the mRNA molecules containing polyA tails. The purified mRNA is fragmented and copied into first strand complimentary DNA (cDNA) using reverse transcriptase and random primers. In a second strand cDNA synthesis step, dUTP replaces dTTP to achieve strand specificity. The final steps add adenine (A) and thymine (T) bases to fragment ends and ligate adapters. The resulting products are purified and selectively amplified for sequencing on NextSeq 550 Illumina system.

The library preparation protocol is shown in the figure 3.8, and 2 Stop Points were observed for the preparation of the library, allowing the division of the protocol into two parts corresponding to two consequently days.

Figure 3.8. Dual-indexed libraries preparation protocol



PART 1. First, messenger RNA with polyA tails was captured by using oligo(dT) magnetic beads. Specifically, 150 ng of total RNA were diluted in a total volume of 25 µL of nuclease-free ultrapure water and then added with 25 µL of RPBX (RNA Purification Beads) and mixed, followed incubation at 65°C for 5 minutes, 4°C for 30 seconds and 23°C for 5 minutes. The reaction product was then washed by using a magnetic stand and a Bead Washing Buffer (BWB), and then eluted by using the magnetic stand and the Elution Buffer (ELB). Once eluted, the mRNA was incubated for 2 minutes at 80°C. The Clean Up mRNA step followed, by binding the mRNA to the beads using the Bead Binding Buffer (BBB), the magnetic stand and subsequently the Bead Washing Buffer. Once the mRNA was cleaned, it followed the Fragmentation and Denaturation mRNA step. The fragmentation consisted of adding the mRNA with 10.5 µL of nuclease-free ultrapure water and 10.5 µL of EPH3 (Elute, Prime, Fragment High Mix) and incubating at 94°C for 8 minutes. Subsequently, the hexamer-primed RNA fragments previously obtained were reverse transcribed to produce first strand complementary DNA (cDNA), by using the First Strand Synthesis Act D Mix, which includes Actinomycin D (FSA), which allows RNA-dependent synthesis and improves strand specificity while preventing spurious DNA-dependent synthesis. The fragmented mRNA was added with 9 µL of FSA and 1 µL of Reverse Transcriptase (RVT) enzyme and incubated as follows: 25°C for 10 minutes, 42°C for 15 minutes, 70°C for 15 minutes.

Subsequently, it followed the Synthesize Second Strand cDNA, which removed the RNA template and synthesized a replacement strand to generate blunt-ended, double-stranded cDNA fragments. In place of deoxythymidine triphosphate (dTTP), deoxyuridine triphosphate (dUTP) was incorporated to quench the second strand

during amplification and achieve strand specificity. The sample was added with 25 µL of Second Strand Marking Master Mix (SMM) and then incubated at 16°C for 1 hour, followed by a clean-up process which consisted in different washes with AMPure XP and ethanol 80%, then resuspended in Resuspension Buffer (RSB). Once resuspended, the samples were stored at -20°C overnight as this presented a safe stopping point as suggested by the manufacturer's instruction.

PART2. Following the first stopping point, it followed the 3'Ends Adenylation step, which consisted in adding an adenine (A) nucleotide to the 3' ends of the blunt fragments to prevent them from ligating to each other during adapter ligation. A corresponding thymine (T) nucleotide on the 3' end of the adapter provides a complementary overhang for ligating the adapter to the fragment. The sample was added with 12.5 µL of A-Tailing mix (ATL4) and then incubated as follows: 37°C for 30 minutes and 70°C for 5 minutes. Subsequently, pre-index anchors were ligated to the ends of the double-stranded cDNA fragments to prepare them for dual indexing (Ligate Anchors step). Each sample was added with 5 µL of unique RNA Index Anchors and 2.5 µL of Ligation Mix (LIGX), then incubated at 30°C for 10 minutes. To stop the ligation, 5 µL of Stop Ligation Buffer (STL) were added to each sample. Once the anchors were added and the ligation stopped, it followed a clean-up procedure with AMPure XP beads and ethanol 80% as previously described. Then, it followed the Amplification of the Library step, which used PCR to selectively amplify the anchor-ligated DNA fragments and add indexes and prime sequences for cluster generation to obtain a dual-indexed library represented by DNA fragments with adapters at each end. In this step, each sample was added with 10 µL of a unique index adapted (labelled ADP0XXX, where the X stands for the specific index from the Index adapter

plate) and 10 µL of Enhanced PCR Mix (EPM); then, the sample was incubated as follows: 30 seconds at 98°C, 6 cycles of 98°C for 10 seconds – 60°C for 30 seconds – 72°C for 30 seconds, and then 72 °C for 5 minutes. Lastly, the dual-index libraries were cleaned up by using the same procedure with AMPure XP beads and ethanol 80% as previously described. Once resuspended in RSB, the dual-indexed libraries were stored at -20°C (for up to 30 days) before proceeding with the quality control as this represented the second safe stopping point suggested by the manufacturer's instructions. All 150 samples were pre-processed for part 1 and part 2 before proceeding with the next steps. The randomized groups are reported in table 3.8.

Table 3.8. Randomization of the 150 samples accordingly to risk groups A, B and C for the pre-processing part 1 and 2.

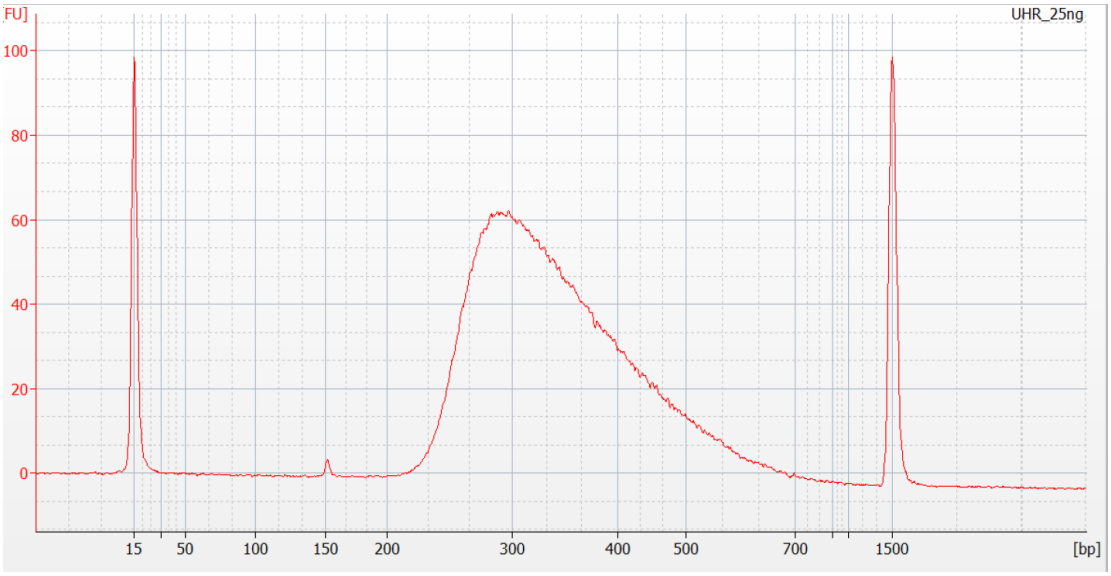
GROUP 1	BR0094PAX	C	GROUP 4	BR0120PAX	B	GROUP 7	BR0092PAX	A
	BR0109PAX	A		BR0060PAX	A		BR0102PAX	A
	BR0020PAX	A		BR0067PAX	B		BR0105PAX	B
	BR0098PAX	A		BR0150PAX	A		BR0101PAX	A
	BR0156PAX	C		BR0059PAX	B		BR0028PAX	C
	BR0043PAX	C		BR0046PAX	C		BR0131PAX	B
	BR0002PAX	C		BR0057PAX	C		BR0078PAX	B
	BR0160PAX	B		BR0115PAX	B		BR0164PAX	B
	BR0155PAX	A		BR0070PAX	A		BR0151PAX	B
	BR0073PAX	C		BR0088PAX	A		BR0133PAX	B
	BR0053PAX	B		BR0100PAX	B		BR0097PAX	A
	BR0089PAX	A		BR0009PAX	C		BR0013PAX	B
	BR0140PAX	A		BR0113PAX	B		BR0090PAX	C
	BR0110PAX	A		BR0146PAX	A		BR0148PAX	B
	BR0169PAX	C		BR0138PAX	A		BR0168PAX	B
	BR0064PAX	C	GROUP 5	BR0071PAX	A	GROUP 8	BR0036PAX	A
	BR0128PAX	B		BR0055PAX	C		BR0022PAX	C
	BR0086PAX	A		BR0024PAX	C		BR0091PAX	C
	BR0111PAX	A		BR0079PAX	B		BR0001PAX	C
	BR0154PAX	A		BR0065PAX	C		BR0127PAX	C
	BR0137PAX	B		BR0005PAX	A		BR0074PAX	A
GROUP 2	BR0173PAX	C		BR0103PAX	C		BR0142PAX	B
	BR0143PAX	A		BR0085PAX	B		BR0050PAX	B
	BR0047PAX	A		BR0118PAX	A		BR0158PAX	B
	BR0018PAX	C		BR0038PAX	C		BR0173PAX	C
	BR0019PAX	C		BR0076PAX	B			
	BR0049PAX	A		BR0124PAX	C			
	BR0135PAX	B		BR0122PAX	C			
	BR0080PAX	B		BR0167PAX	C			
	BR0144PAX	B		BR0121PAX	A			
	BR0114PAX	B		BR0084PAX	B			
	BR0145PAX	B		BR0093PAX	A			
	BR0083PAX	C		BR0116PAX	A			
	BR0119PAX	A		BR0006PAX	C			
	BR0045PAX	C		BR0039PAX	C			
	BR0072PAX	A		BR0129PAX	A			
GROUP 3	BR0033PAX	A	GROUP 6	BR0153PAX	B			
	BR0077PAX	A		BR0161PAX	B			
	BR0163PAX	C		BR0081PAX	B			
	BR0099PAX	B		BR0108PAX	A			
	BR0165PAX	C		BR0149PAX	A			
	BR0162PAX	B		BR0087PAX	B			
	BR0125PAX	A		BR0021PAX	C			
	BR0032PAX	C		BR0061PAX	C			
	BR0095PAX	A		BR0130PAX	A			
	BR0171PAX	C		BR0075PAX	A			
	BR0066PAX	A		BR0134PAX	C			
	BR0051PAX	B		BR0123PAX	C			
	BR0096PAX	A		BR0056PAX	C			
	BR0152PAX	B		BR0027PAX	C			
	BR0106PAX	B		BR0035PAX	B			
	BR0040PAX	C		BR0139PAX	B			
	BR0037PAX	A		BR0117PAX	B			
	BR0054PAX	B		BR0172PAX	C			
	BR0132PAX	C		BR0058PAX	C			
	BR0044PAX	C		BR0112PAX	A			
	BR0104PAX	A		BR0107PAX	A			
	BR0170PAX	C	GROUP 7	BR0157PAX	B			
	BR0052PAX	B		BR0147PAX	B			
	BR0015PAX	C		BR0048PAX	A			
	BR0082PAX	B		BR0030PAX	C			
	BR0029PAX	C		BR0069PAX	A			
	BR0141PAX	B		BR0136PAX	B			

3.7.2 Dual-indexed libraries quality control check

This step checks the concentration and quality of the final libraries. The quality check of the libraries was performed by analysing 1 µL library using the Agilent 2100 Bioanalyzer and DNA 1000 Kit, whereas the concentration was measured by analysing 2 µL of the library using the Qubit dsDNA BR Assay kit.

A good quality library should show a peak around 300 bp and the absence of the dimers peak at around 150 bp in the Bioanalyzer Trace. If the latter is visible, it is recommended to perform an additional clean up step with AMPure XP beads 0.8X and ethanol 80% as previously described. An example of a good library is represented in figure 3.9.

Figure 3.9. Example Bioanalyzer Trace. From Illumina Stranded mRNA Prep Ligation Reference Guide.



3.7.3 Library dilution to the starting concentration

Once the quality of the libraries was checked, it followed the dilution of the libraries to the starting concentration for the NextSeq 550 System, which was 1 nM. For calculating the molarity value of each library, the following formula was followed:

$$\frac{ng/\mu L}{660\frac{6}{mol}x\ average\ library\ size}\ x\ 10^6\ = Molarity\ (nM)$$

Each library was diluted to the starting concentration of 1nM and then 10 µL of each diluted library was combined in a tube to pool libraries.

3.7.4 Library denaturation

To denature the library, 20 µL of 1 nM pool library were combined with 20 µL 0.2 N NaOH, vortexed and incubated for 5 minutes. Subsequently, the solution was added with 20 µL of 200 mM Tris-HCl pH 7, vortexed and centrifuged. This denatured library was then dilute to a 20 pM concentration by adding 940 µL of HT1 buffer. To obtain the final 1.4 pM library for High Outputs kits, the denatured 20 pM library solution was diluted as follows: 91 µL denatured library solution and 1209 µL HT1.

Each 1.4 pM library was added with 1.4 pM PhiX Control as follows: 13 µL of denatured and diluted PhiX control 1.4 pM and 1287 µL of denatured and diluted library 1.4 pM. Once combined, the final library was placed on ice until the loading on the High Output Cartridge.

3.7.5 Sequencing

The sequencing run was performed on the NextSeq 550 Illumina platform, by using High Output Kit v2.5 (150 Cycles) paired-ended, read length 74. Cluster generation and

sequencing were performed on the instrument. During cluster generation, single DNA molecules were bound to the surface of the flow cell, and then amplified to form clusters. Clusters were imaged using two-channel sequencing chemistry and filter combinations specific to each of the fluorescently labelled chain terminators. After the imaging of a tile on the flow cell was completed, the next tile was imaged. The process was repeated for each cycle of sequencing. Following image analysis, the software performed base calling, filtering, and quality scoring.

As the run progresses, the control software automatically transfered base call (BCL) files to BaseSpace Sequence Hub, Local Run Manager, or another specified output location for secondary analysis. After the run, an instrument wash began automatically using components already loaded on the instrument.

The sequencing was performed following the NextSeq 550 System Guide (document #15069765,

https://support.illumina.com/content/dam/illuminasupport/documents/documentation/system_documentation/nextseq/nextseq-550-system-guide-15069765-06.pdf).

Before library dilution and sequencing, samples were randomized to allow the runs of 14 samples per run. The randomized groups for the sequencing steps are reported in table 3.9.

Table 3.9. Randomization of the 150 samples accordingly to risk groups A, B and C for the sequencing step on the array

BR0094PAX	C	RUN 1	BR0125PAX	A	RUN 4	BR0085PAX	B	RUN 7	BR0030PAX	C	RUN 10
BR0109PAX	A		BR0032PAX	C		BR0118PAX	A		BR0069PAX	A	
BR0020PAX	A		BR0095PAX	A		BR0038PAX	C		BR0136PAX	B	
BR0098PAX	A		BR0171PAX	C		BR0076PAX	B		BR0092PAX	A	
BR0156PAX	C		BR0066PAX	A		BR0124PAX	C		BR0102PAX	A	
BR0043PAX	C		BR0051PAX	B		BR0122PAX	C		BR0105PAX	B	
BR0002PAX	C		BR0096PAX	A		BR0167PAX	C		BR0101PAX	A	
BR0160PAX	B		BR0040PAX	C		BR0121PAX	A		BR0028PAX	C	
BR0155PAX	A		BR0037PAX	A		BR0084PAX	B		BR0131PAX	B	
BR0073PAX	C		BR0054PAX	B		BR0093PAX	A		BR0078PAX	B	
BR0053PAX	B		BR0132PAX	C		BR0116PAX	A		BR0164PAX	B	
BR0089PAX	A		BR0170PAX	C		BR0006PAX	C		BR0151PAX	B	
BR0140PAX	A		BR0052PAX	B		BR0039PAX	C		BR0133PAX	B	
BR0110PAX	A		BR0015PAX	C		BR0129PAX	A		BR0097PAX	A	
BR0169PAX	C	RUN 2	BR0082PAX	B	RUN 5	BR0153PAX	B	RUN 8	BR0013PAX	B	RUN 11
BR0064PAX	C		BR0141PAX	B		BR0161PAX	B		BR0090PAX	C	
BR0128PAX	B		BR0060PAX	A		BR0081PAX	B		BR0148PAX	B	
BR0086PAX	A		BR0067PAX	B		BR0108PAX	A		BR0168PAX	B	
BR0111PAX	A		BR0150PAX	A		BR0149PAX	A		BR0127PAX	C	
BR0154PAX	A		BR0059PAX	B		BR0087PAX	B		BR0074PAX	A	
BR0137PAX	B		BR0057PAX	C		BR0021PAX	C		BR0142PAX	B	
BR0143PAX	A		BR0115PAX	B		BR0061PAX	C		BR0050PAX	B	
BR0047PAX	A		BR0070PAX	A		BR0130PAX	A		BR0158PAX	B	
BR0018PAX	C		BR0088PAX	A		BR0075PAX	A		BR0173PAX	C	
BR0019PAX	C		BR0100PAX	B		BR0036PAX	A		BR0138PAX	A	
BR0049PAX	A		BR0009PAX	C		BR0022PAX	C				
BR0135PAX	B		BR0113PAX	B		BR0091PAX	C				
BR0080PAX	B		BR0146PAX	A		BR0001PAX	C				
BR0144PAX	B	RUN 3	BR0044PAX	C	RUN 6	BR0134PAX	C	RUN 9			
BR0114PAX	B		BR0104PAX	A		BR0123PAX	C				
BR0145PAX	B		BR0029PAX	C		BR0056PAX	C				
BR0083PAX	C		BR0120PAX	B		BR0027PAX	C				
BR0119PAX	A		BR0046PAX	C		BR0035PAX	B				
BR0045PAX	C		BR0071PAX	A		BR0139PAX	B				
BR0072PAX	A		BR0055PAX	C		BR0117PAX	B				
BR0033PAX	A		BR0024PAX	C		BR0172PAX	C				
BR0077PAX	A		BR0079PAX	B		BR0058PAX	C				
BR0163PAX	C		BR0065PAX	C		BR0112PAX	A				
BR0099PAX	B		BR0005PAX	A		BR0107PAX	A				
BR0165PAX	C		BR0152PAX	B		BR0157PAX	B				
BR0162PAX	B		BR0106PAX	B		BR0147PAX	B				
			BR0103PAX	C		BR0048PAX	A				

3.8 FASTQ QA/QC

Raw data from RNA Sequencing are represented by FastQ, which is a text file containing the sequence data from the clusters that pass filter on a flow cell. Before proceeding with the biostatistical analysis, the quality of the data was checked by using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/), which is a quality control tool for high throughput sequence data. Modern high throughput sequencers generate hundreds of millions of sequences in a single run and for this reason, performing quality controls checks before performing subsequent analysis is necessary to ensure good quality of raw data and to exclude problems or biases in the data. FastQC aims to provide a QC report that can spot problems which originate either in the sequencer or in the starting library material. The FastQC quality checks provided are:

- Basic Statistics: it generates some simple composition statistics for the file analysed (file name, file type, encoding, total sequences, filtered sequences, sequence length and %GC)
- Per Base Sequence Quality: it shows an overview of the range of quality values across all bases at each position in the FastQ file. Figure 3.10 represents an example of a Box Whisker plot.
- Per Sequence Quality Scores: it allows to see whether a subset of the sequences have universally low-quality values (Figure 3.11).
- Per Base Sequence Content: it plots the proportion of each base position in a file for which each of the four normal DNA bases has been called. In a random library, little or no differences are expected between the different bases of a sequence run, so the lines in this plot should run parallel with each other. The relative

amount of each base reflects the overall amount of these bases in our genome. Figure 3.12 represents an example of the Per Base Sequence plot.

- Per Sequence GC Content: it measures the GC content across the whole length of each sequence in a file and compares it to a modelled normal distribution of GC content. In a random library, a normal distribution of GC content is expected, with a central peak corresponding to the overall GC content of the underlying genome (Figure 3.13).
- Per Base N Content: If the sequencer is unable to make a base call with sufficient confidence, it will substitute an N rather than a base call. This module plots the percentage of base calls at each position for which an N was called (Figure 3.14).
- Sequence Length Distribution: it generates a graph showing the distribution of fragment sizes in the file which was analysed (Figure 3.15).
- Duplicate Sequences: it counts the degree of duplication for every sequence in a library and creates a plot showing the relative number of sequences with different degree of duplication (Figure 3.16).
- Overrepresented Sequences: it lists all the sequence which are overrepresented, which means that make up more than 0,1% of the total.
- Adapter content: this is a Kmer Content module that does a generic analysis of all the Kmers in the library to identify those which do not have coverage through the lengths of the reads.
- Kmer Content
- Per Tile Sequence Quality: it allows to look at the quality scores for each tile across all the bases to see if there is a loss in quality associated with only one part of the flow cell.

For each of these parameters, FastQC raise “warning” or “error” signs to immediately inform and understand whether an error occurred for one or more parameters.

Figure 3.10. Per Base Sequence Quality Box Whisker

The central red line is the median value; the yellow box represents the inter-quartile range (25-75%); the upper and lower whiskers represent the 10% and 90% points; the blue line represents the mean quality. The y-axis on the graph shows the quality scores. The higher the score the better the base call. The background of the graph separates the y-axis into very good quality (green) calls, reasonable quality (orange) calls, and poor quality (red) calls. The quality of the calls on most sequencing platforms will degrade as the run progresses, so it is common to see the yellow boxes falling into the orange area (lower quality) towards the right of the x-axis, representing the end of a read.

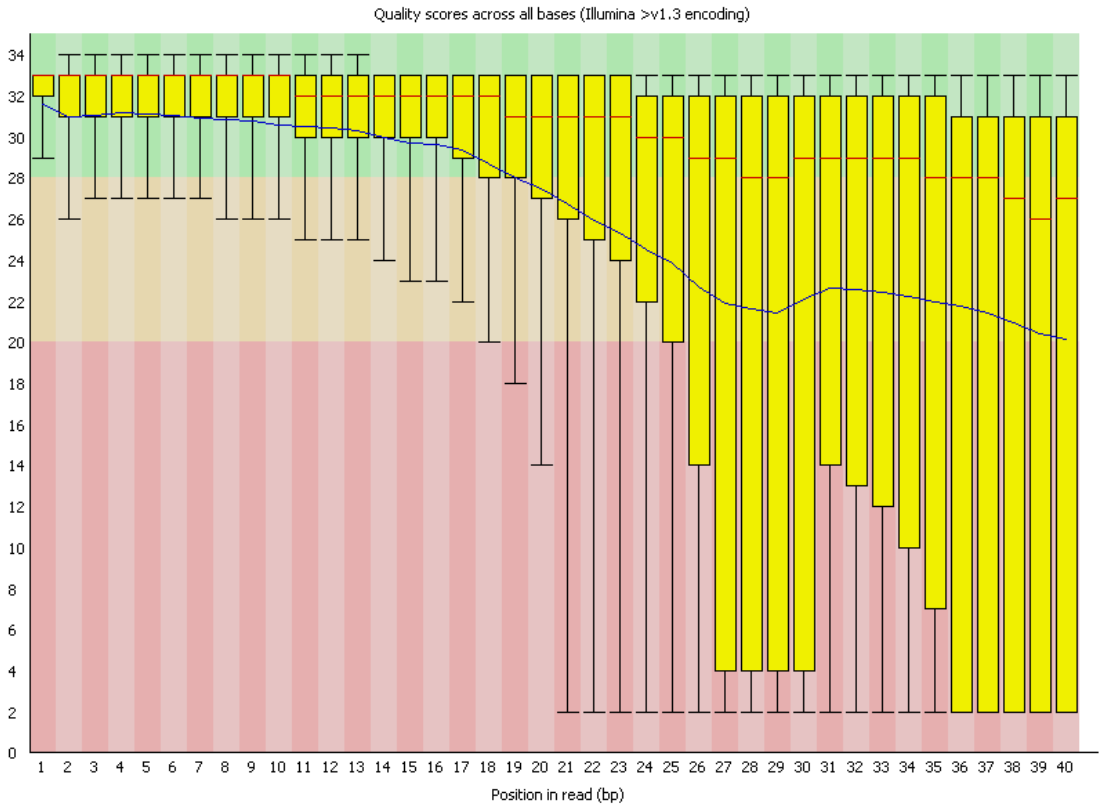


Figure 3.11. Per Sequence Quality Scores Graph

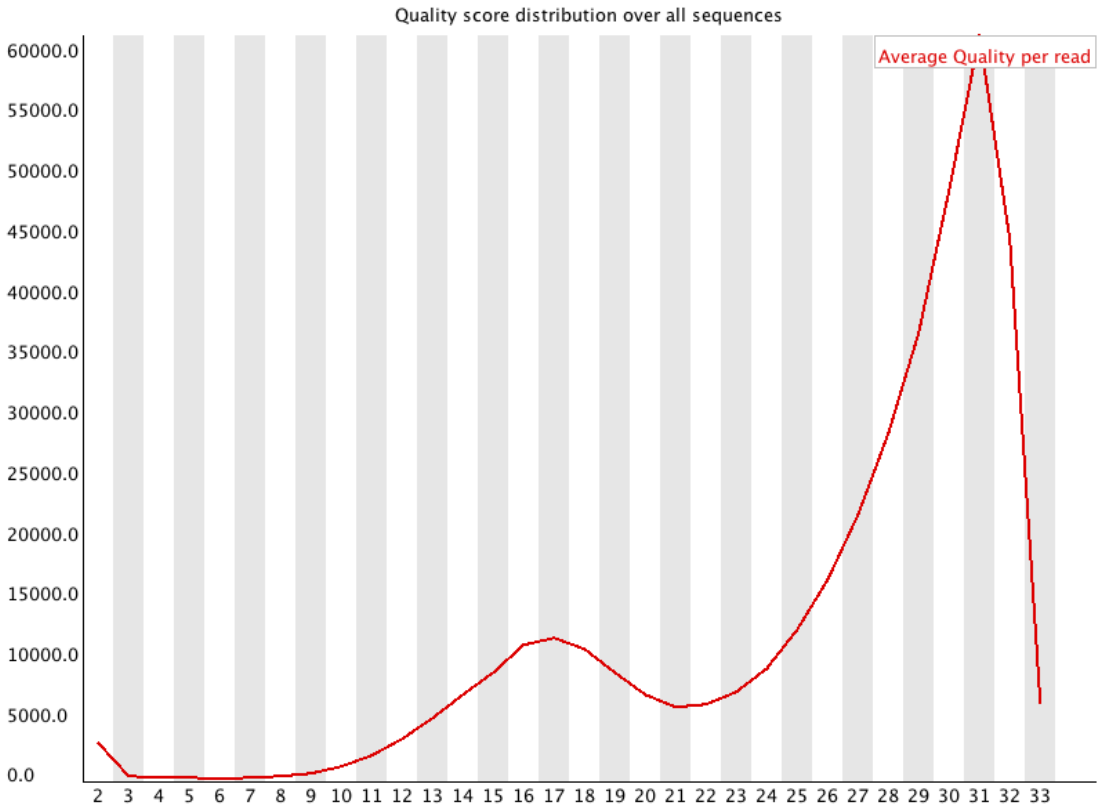


Figure 3.12. Per Base Sequence Content

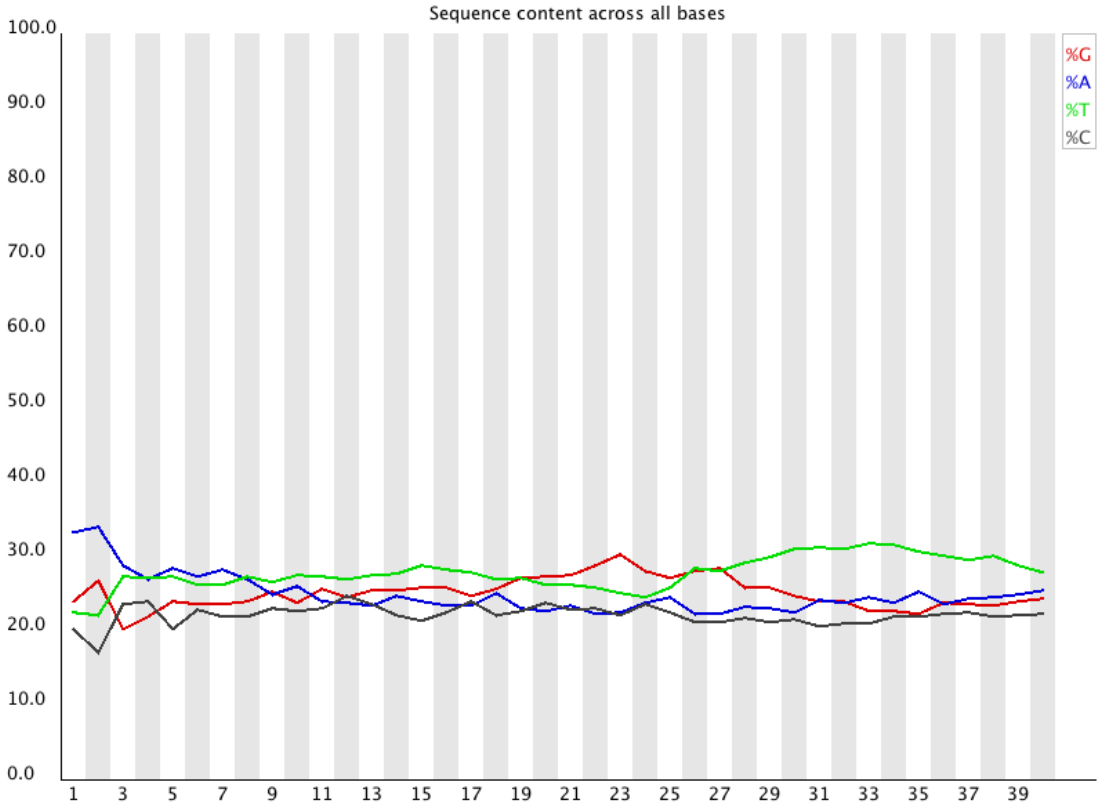


Figure 3.13. Per Sequence GC Content Graph

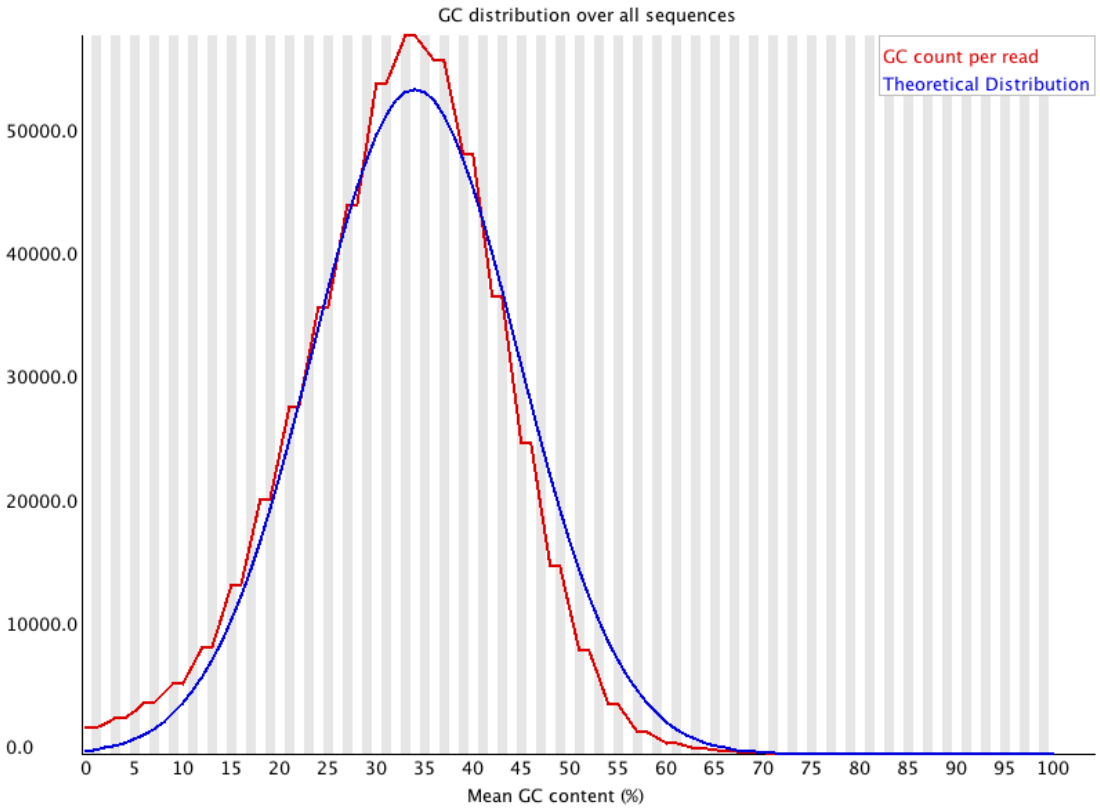


Figure 3.14. Per Base N Content Graph

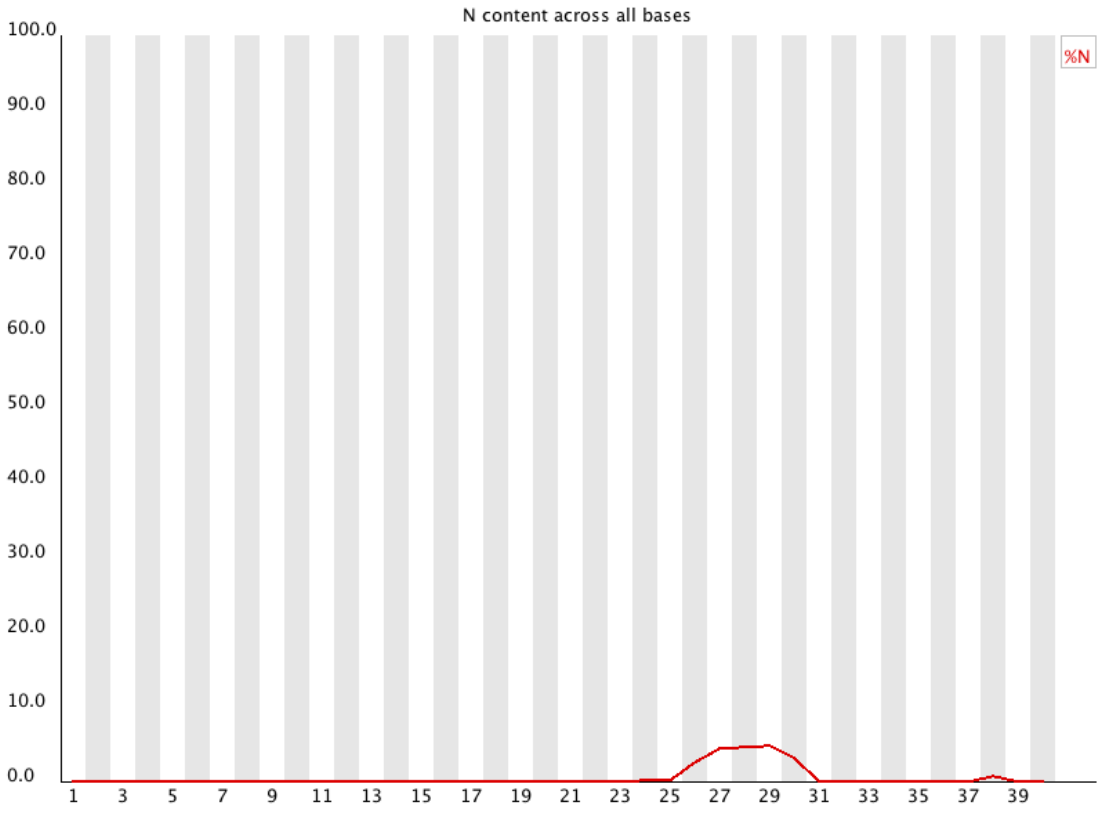


Figure 3.15. Sequence Length Distribution Graph

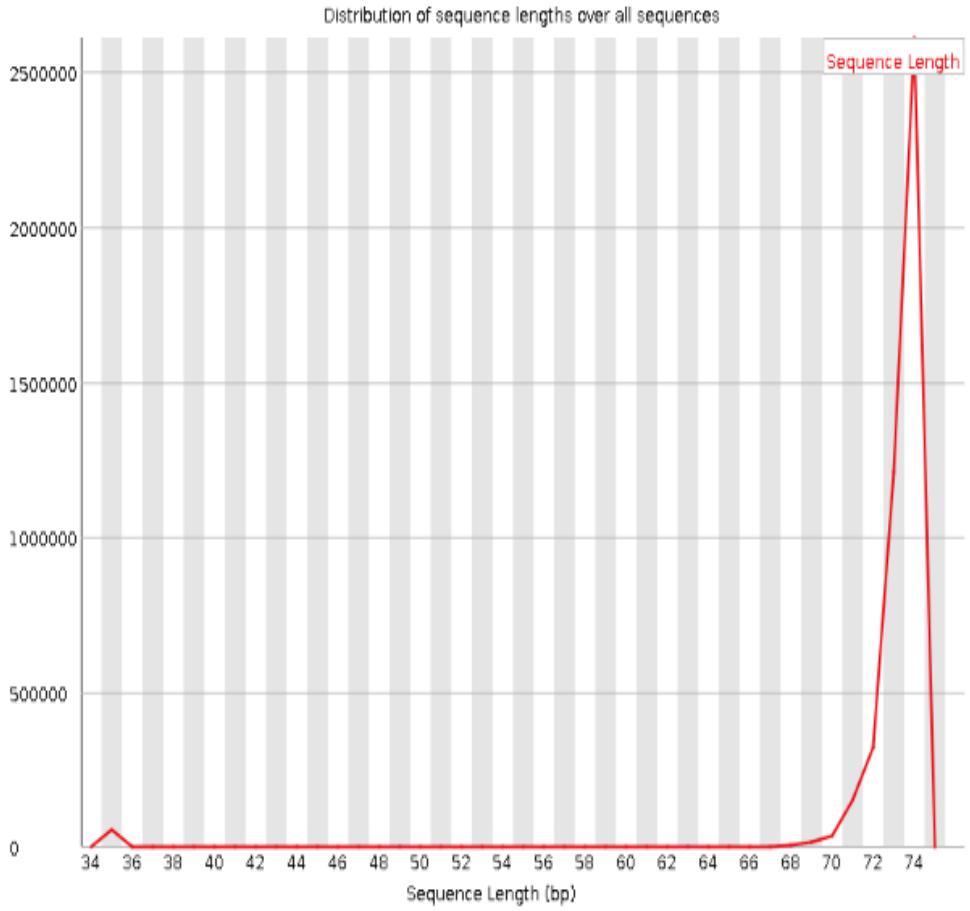
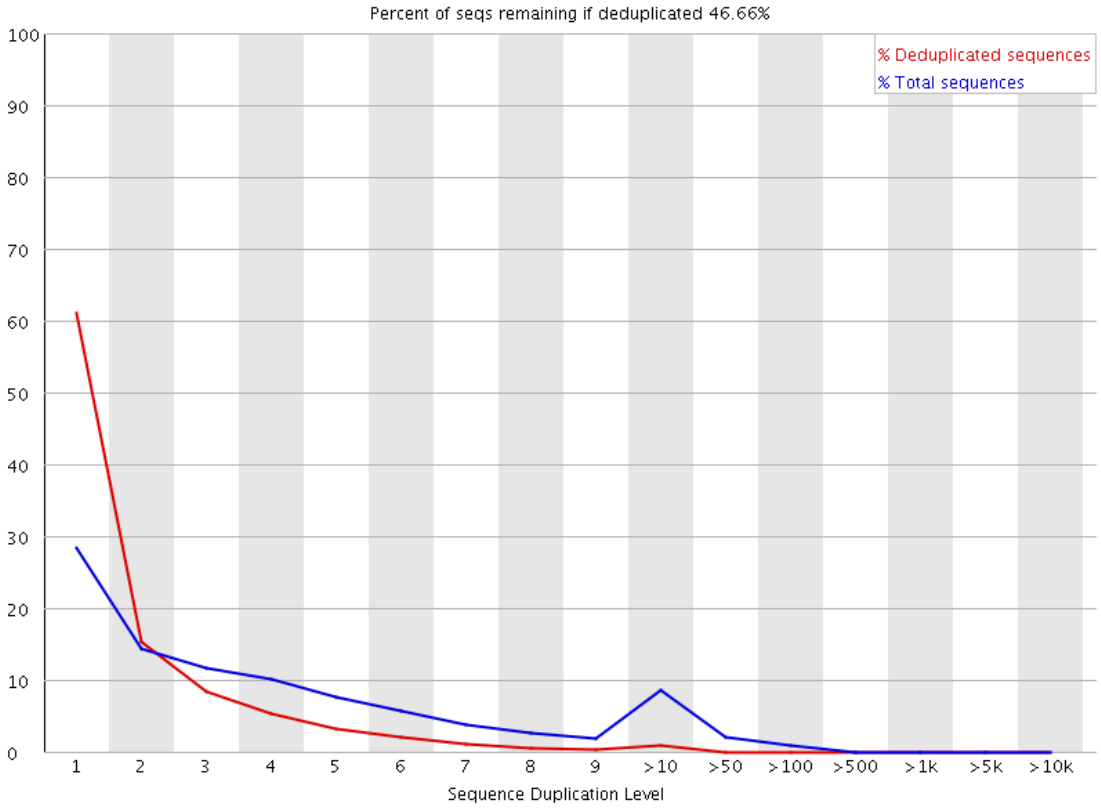


Figure 3.16. Duplicate Sequences Graph



3.9 RNA-Seq biostatistical analysis

The raw read counts were quantified at the transcript level by using Salmon (v 1.4.0) which is a tool for fast transcript quantification from RNA-seq data that allows to accurately quantify transcripts without applying reads alignment. Salmon was used in quasi-mapping mode which consisted of two steps: i) indexing of the transcriptome and, ii) quantification of the set of raw sequencing reads (FASTQ format) (Patro, Duggal, Love, Irizarry, & Kingsford, 2017). The first step was done by building a decoy-aware transcriptome where the entire human genome was concatenated to the end of the human transcriptome and was subsequently indexed with the chromosome names following the instructions reported in https://combine-lab.github.io/alevin-tutorial/2019/selective-alignment/. Both genome and transcriptome (Release 38 (GRCh38.p13)) were downloaded from https://www.gencodegenes.org/human/. The second step consisted in the quantification of the paired-end reads directly against the index built during the previous step.

Transcript-level differential expression was assessed using DESeq2 (v1.30.1) in R to compare the three risk groups divided or not by sex (MDD *vs* HR; MDD *vs* LR; HR *vs* LR; for males: MDD *vs* HR; MDD *vs* LR; HR *vs* LR. For females: MDD *vs* HR; MDD *vs* LR; HR *vs* LR), accounting for RNA-Seq run as covariate. Before differential expression analysis, the transcript set was filtered for library size, and transcripts with less than 10 counts across all samples were excluded. Differentially expressed genes were identified by applying a FC cut-off of ± |1.2|, an unadjusted p-value < 0.05 and a Benjamini-Hochberg adjusted false discovery rate (FDR) cut-off of 0.05 (q-value).

Genes differently modulated across groups were then imported in Ingenuity Pathway Analyses Software (IPA) to identify associated biological pathways.

3.10 Correlation between microarrays and RNA-Seq

To identify common results between microarray and RNA-Seq results, a Spearman's correlation was computed by using SPSS Software. Specifically, the correlation analysis was performed considering the following comparison: MDD *vs* HR, MDD *vs* LR, and HR *vs* LR considering only the entire cohort and not the results divided for biological sex. The Spearman's correlation was computed using as input values the relative raw expression profile of the common genes between both platforms, meaning the fold-changes value for each common gene. For each of the three comparisons previously mentioned, the correlation was performed on the genes in common obtained without applying any cut-offs filter (neither p-value or FC), and on the genes in common between the two platforms and surviving the p-value of 0.05. Genes in common between microarrays and RNA-Seq were obtained by overlapping in a Venn diagram the list of genes (accordingly to the presence or not of the cut-offs) from the two platforms.

4. Results

In this section, I will present the results following the order reported in the Methods' section. I will firstly describe the sociodemographic and clinical characteristics of the IDEA-RiSCo sample constituted by 150 adolescents. Then, I will present the data from the quality control of the RNA samples extracted. I will then show the results of the genome-wide gene expression analysis by using the microarrays of Gene-Atlas Affymetrix Platform. I will firstly show the quality control results of the Affymetrix technique, by reporting both the results of the internal controls of the Gene-Atlas platform and the quality controls of the raw data (CEL files) performed by using Partek Genomic Suite Software. I will then show the biostatistical analysis performed with Partek Genomic Suite in terms of genes differently expressed (DEGs) and the results of the pathways analysis performed by using Ingenuity Pathways Analysis Software.

In the second main part of the results, I will describe the data from the second genome-wide approach using RNA Sequencing with NextSeq550 Platform. Similarly, to the structure previously described for Affymetrix microarrays, I will firstly report the quality controls analysis of the FASTQ raw data performed via FastQC. Then I will show the biostatistical analysis performed via Salmon and DESeq2, by showing the DEGs and the results of the pathways analysis performed by using Ingenuity Pathways Analysis Software, as previously described in paragraph 3.6.2.

Lastly, I will report the results related to the correlation analysis of the DEGs in common between microarrays and RNA-Seq.

4.1 IDEA-RiSCo

4.1.1 Sociodemographic and clinical characteristics of adolescents included in the RiSCo cohort

Fifty adolescents were recruited for each group (MDD, HR or LR) with 25 girls and 25 boys in each group, for a total of 75 females and 75 males in the whole cohort. The basic sociodemographic and clinical characteristics of the adolescents are reported in table 4.1. Mean (± SD) of age was 15.4 ± 0.8 years for the LR group, 15.8 ± 0.8 for HR group, and 15.8 ± 0.7 for MDD adolescents. Thus, there was a small but significative difference among groups, with the LR group being slightly younger than both HR and MDD. No significant differences among groups were reported for body mass index (mean ± SD), showing LR group mean 22.1 ± 5.5 kg/m^2, HR 22.4 ± 4.8 kg/m^2, and MDD 22.7 ± 3.9 kg/m^2. Similarly, no differences between groups were observed also for body temperature.

The mean PHQ-A score was 9.52, with higher scores observed in girls (11.51 compared with 7.07 in boys). The PHQ-A and the IDEA-RS for both boys and girls are shown in figure 4.1. The IDEA-RS sociodemographic parameters scores for the IDEA-RiSCo are reported in table 4.2. No significant differences were identified in the proportion of the adolescents who self-identified as having white skin color among the three group. Involvement in fights, school failures and drug use were less common in LR adolescents than in both HR and MDD group. Adolescents with depression reported higher rates of history of running away from home compared with HR and LR groups. The relationship with fathers and both parents was reported as more positive by LR adolescents than their peers in HR and MDD groups. A stepwise decrease from LR to

HR to MDD was reported with regards to the relationship with the mother as well as in the meeting with friends. In terms of maltreatment, all adolescents in the LR were categorized in the “no maltreatment” group, whereas the majority from HR group and almost all from MDD group were classified as having experiences of “severe maltreatment”. These results are in line with the CTQ score previously reported in table 4.2, as it was observed a stepwise increase of the CTQ mean scores from LR to HR to MDD, respectively 29.16 ± 3.3, 38.08 ± 8.2 and 51.56 ± 13.2.

Table 4.1. Phenotypic characteristics of the IDEA-RiSCo sample

	Low Risk **Mean (SD)**	**High Risk** **Mean (SD)**	**MDD** **Mean (SD)**
Age (years)	15.4 (0.8)	15.8 (0.8)	15.8 (0.7)
Body Mass Index	22.1 (5.5)	22.4 (4.8)	22.7 (3.9)
Body Temperature	35.9 (5.5)	36.0 (0.5)	36.1 (0.6)
IDEA-RS (%)	1.33 (0.3)	8.21 (1.6)	9.24 (5.6)
PHQ-A	2.82 (1.5)	3.96 (1.6)	18.82 (4.5)
CTQ	29.16 (3.3)	38.08 (8.2)	51.56 (13.2)

Figure 4.1. PHQ-A score and Probability of Depression in 3 years of adolescents screened at schools and included in the IDEA-RiSCo, boys and girls.

Vertical dotted lines show the Patient Health Questionnaire—adolescent version (PHQ-A) cut-offs, and horizontal dotted lines show the Identifying Depression Early in Adolescence risk score (IDEA-RS) cut-offs. Low risk (LR) adolescents appear in the lower left quadrant (PHQ-A≤6 and IDEA-RS≤20th percentile); high risk (HR) adolescents in the upper left quadrant (PHQ-A≤6 and IDEA-RS≥90th percentile); and adolescents with current major depressive disorder (MDD), in the upper right quadrant (PHQ-A≥10 and IDEA-RS≥90th percentile). Gray dots representing the students who did not meet inclusion criteria are spread over all quadrants. From (Kieling et al., 2021).

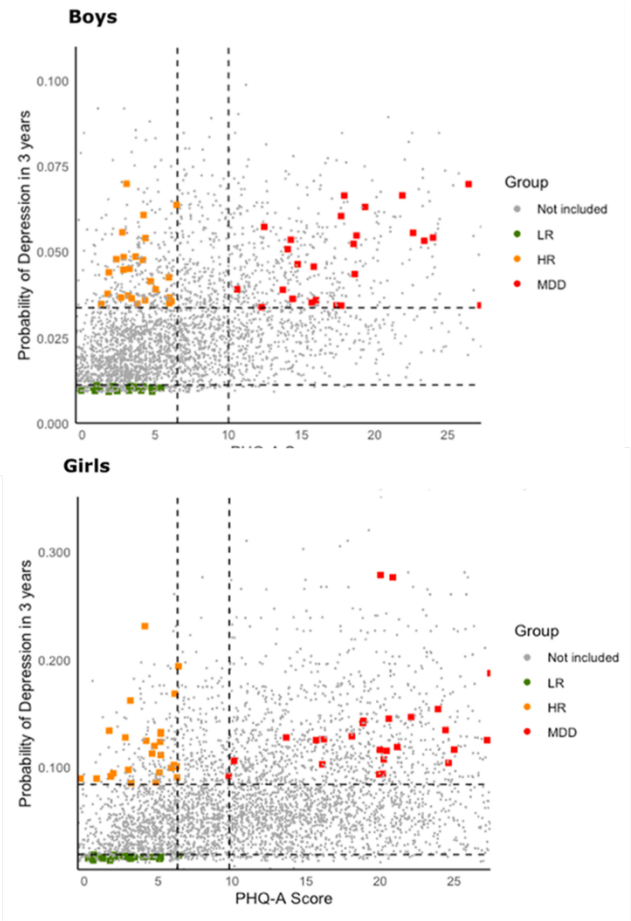


Table 4.2. IDEA-RS sociodemographic variables in the IDEA-RiSCo.

"Relationship" variables were analyzed as continuous (mean, SD), with answers ranging from 1 (bad) to 5 (great).

	Low Risk n (%)	**High Risk n (%)**	**MDD n (%)**
Sex, female	25 (50.00)	25 (50.00)	25 (50.00)
Skin color, non-white	22 (44.00)	26 (52.00)	26 (52.00)
Meets friends	49 (98.00)	40 (80.00)	30 (60.00)
School Failure	0 (0.00)	29 (58.00)	25 (50.00)
Ran away	1 (2.00)	3 (6.00)	13 (26.00)
Any drug use	29 (58.00)	44 (88.00)	47 (94.00)
Fights	0 (0.00)	20 (40.00)	27 (54.00)
Relationship with father (mean, SD)	4.52 (0.79)	2.48 (1.22)	2.00 (1.18)
Relationship with mother (mean, SD)	4.78 (0.54)	3.92 (1.01)	3.14 (1.14)
Relationship between parents (mean, SD)	4.18 (1.08)	2.38 (1.23)	1.94 (1.04)
Childhood Maltreatment - None	50 (100.00)	1 (2.00)	0 (0.00)
Childhood Maltreatment - Probable	0 (0.00)	12 (24.00)	4 (8.00)
Childhood Maltreatment - Severe	0 (0.00)	37 (74.00)	46 (92.00)

4.2 Quality Control of RNA samples

Following the RNA extraction from Paxgene blood tubes, RNA quantity and quality were assessed evaluating the A260/280 and A260/230 ratios using a Nanodrop 2000 spectrophotometer. One µl of each sample was quantified by using the Nanodrop and the mean concentration (± SD) of all the 150 samples was 178.16 ng/µl ± 61.08 ng/µl, with a minimum concentration of 53.9 ng/µl and a maximum concentration of 380.5 ng/µl. By using Nanodrop, the absorbance at 260 nm and 280 nm was measured, as well as the 260/280 and 260/230 ratios for all the 150 samples and all the values are reported in Appendix A, Table A.

Further quality control analysis was performed by using the Agilent Bioanalyzer 2100, and the RNA Integrity Number (RIN) was obtained for all the samples. The RIN indicates the RNA intactness by evaluation of the ribosomal ratio (18S and 28S) and it is based on a numbering system from 1 to 10, with 1 indicating a degraded profile and 10 the most intact (https://www.agilent.com/cs/library/applications/5989-1165EN.pdf); for genome-wide gene expression analysis, RIN values are considered acceptable within the range 7-10. In the assessed samples, I obtained and average (±SD) RIN value of 9.2 ± 0.5, with a maximum RIN of 10 and a minimum of 7.2. In particular, out of 150 samples, 96 samples reported a RIN value > 9; 43 samples had 8 < RIN < 9; 7 samples reported 7 < RIN < 8, and for 4 out of 150 samples' RIN was not detectable. The RIN of all the 150 samples is reported in Appendix B, Table B.

4.3 Affymetrix microarrays – Gene Atlas Platform

4.3.1 Gene Atlas Affymetrix QA/QC

The Gene Atlas Affymetrix instrument has quality control processes for gene expression analysis, to enable the user to check the data quality. These QA/QC evaluates hybridization controls, labelling controls and internal controls genes. For each sample, once the imaging phase is completed, a QC Report Summary is available. Specifically, the QC checks are the following:

- Summary
- Signal
- Hybridization Controls
- Labelling controls
- Sample quality

Signal Value: it is a measure of the average brightness of the probe sets on the array, minus the background noise. The Signal to Noise ratio must be above a certain value for the array to pass QC.

Hybridization Controls: Biotin labelled controls which are added to the hybridization cocktail. The 20X Eukaryotic Hybridization controls are high-quality controls for monitoring array hybridization, washing, and staining for reproducible results. The 20X Eukaryotic Hybridization Controls are composed of a mixture of biotinylated and fragmented aRNA of bioB, bioC, and bioD from *E. coli* in staggered concentrations. The 20X Eukaryotic Hybridization Controls are spiked into the hybridization cocktail,

independent of RNA sample preparation, and are thus used to evaluate sample hybridization efficiency on eukaryotic gene expression arrays.

Labelling Controls: Poly A Controls are added to the RNA Sample prior to using IVT express kit. Four independent poly-A RNA controls, derived from the lys, phe, and dap genes of *B. subtilis*, are provided conveniently in a pre-mixed stock solution at staggered concentrations. After spiking directly into eukaryotic total RNA samples, labelled aRNA targets are prepared and hybridized onto GeneChip expression arrays. The resultant signal intensities for the poly-A RNA controls serve as sensitive indicators of the efficiency of the labelling reaction and are independent of input sample RNA quality.

Sample Quality: It refers to housekeeping genes, which are gene transcripts that are constitutively expressed on most samples. These transcripts serve as internal controls, are useful for monitoring the quality of the starting sample and are subject to any variability in the labelling of the sample and hybridization for the array. For human, GAPDH is used to assess RNA sample and assay quality. The signal values for the 3' probe sets are compared with the signal values for the corresponding 5' probe sets. If the ratio is greater than 3, the sample is failed.

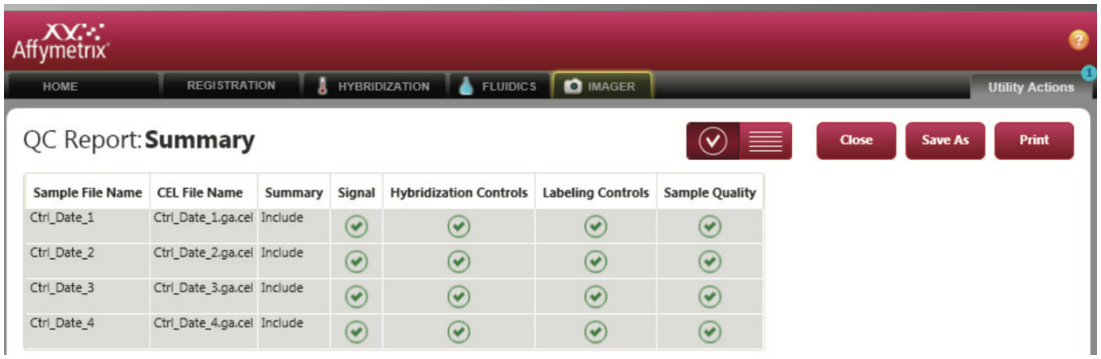
The summary indicates if the sample meets the QC thresholds, and the results are the following:

- Include: Sample data meets QC thresholds
- Exclude: Sample data does not meet the QC thresholds.

The exclusion of a samples is due to an issue occurring in one of the other parameters, which can be labelled with a green tick (positive QC) or red cross (negative QC). See figure 4.2 for an example of the QC Report table.

The QC report summary was evaluated for each of the 150 samples after the imaging process and all samples passes the quality controls and were labelled as “included” and there was no need to re-process any of the 150 samples. The QC Report of all the 150 samples (divided in 4 samples per imaging process, accordingly to the randomization) is reported in Appendix C, Table C.

Figure 4.2. Example of a QC Report Summary (from geneatlas_setup_verification_manual)



4.3.2 Partek Genomic Suite QA/QC

The Software Partek Genomic Suite provides QA/QC report after importing the raw data (CEL file) and adding the samples attributes (for this analysis: risk group and sex). The QA/QC report provided by Partek Genomic Suite are:

- PCA Scatter Plot
- Sample Histogram
- Sample Box & Whiskers Chart

The PCA allows to visualize the general distribution of each sample; each point represents a sample, and they are colored accordingly to a selected variable. The PCA is an example of exploratory data analysis, and it allows to identifying outliers as well as possible major effect in the data.

Figure 4.3 reports the PCA accordingly to the scan date; it can be observed that there is not a clear separation of the samples accordingly to the scan date; therefore, a batch effect or an effect due to the scan date was excluded.

Figure 4.4 reports the PCA accordingly to the risk groups (MDD; HR; LR). This PCA did not show clear separation among the groups, suggesting no major separation according to risk groups.

Figure 4.5 reports the PCA according to biological sex (males and females).

The histogram plots one line for each of the samples with the intensity of the probes graphed on the x-axis and the frequency of the probe intensity on the y-axis. This allows to visualize the distribution of the intensities to identify any outliers.

Similarly to the PCA, it is possible to visualize the results by colour each sample accordingly to each variable (Figure 4.6 and 4.7 coloured for scan date and risk group respectively).

The absence of outliers or of a batch effect in this dataset, was identified by the fact that all the single histograms followed the same distribution, and no secondary peaks or other distributions were observed for any of the 150 samples.

In the Sample Box & Whiskers Chart, each box is a sample, and the boxes are coloured accordingly to the selected variable (Figure 4.8 and 4.9 coloured for scan date and risk group respectively). The intensity of the probes is graphed on the y-axis, the line inside the box represents the median of the intensities (2^{nd} quartile), whereas the box represents the first and third quartiles.

Figure 4.3. Partek Genomic Suite PCA by Scan Date

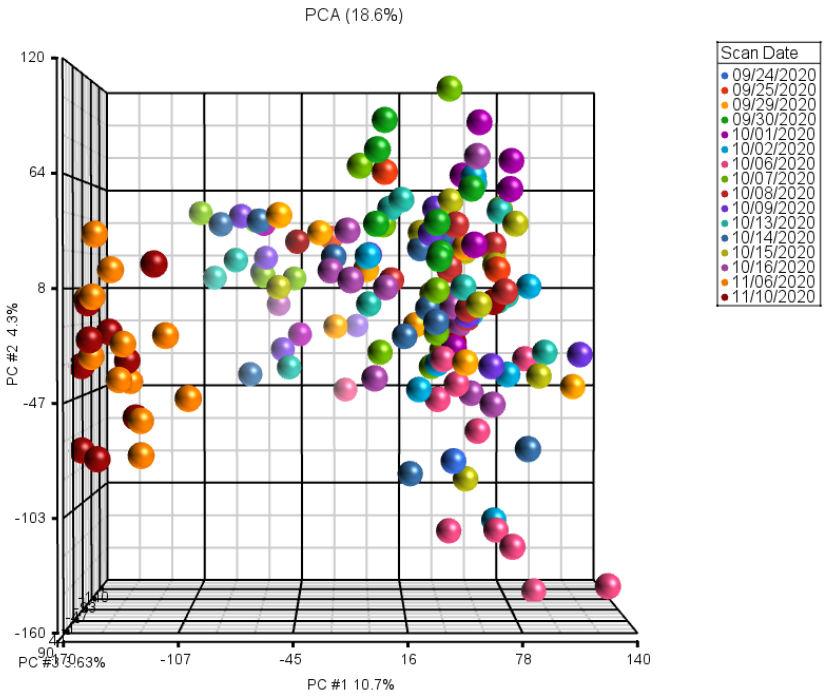


Figure 4.4. Partek Genomic Suite PCA by Risk Group

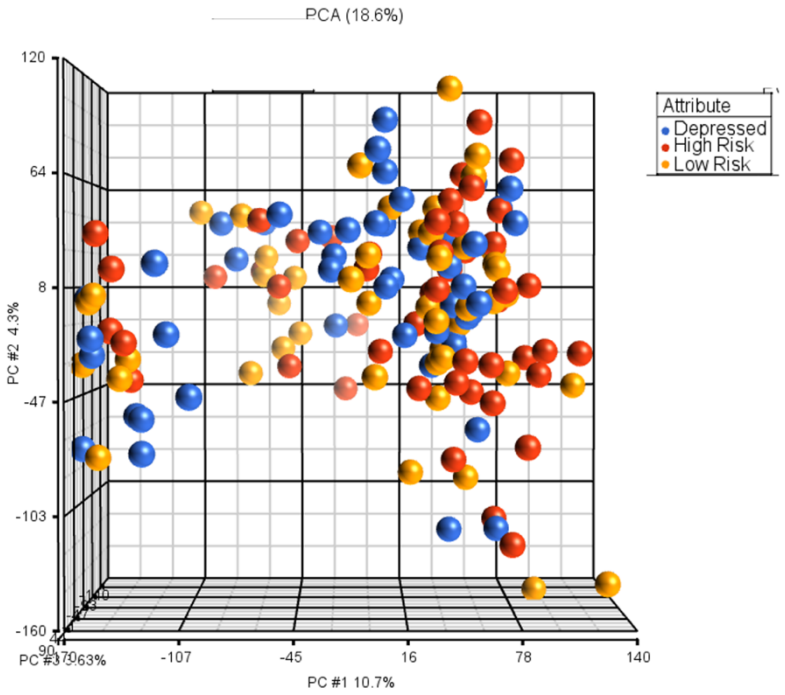


Figure 4.5. Partek Genomic Suite PCA by Biological Sex

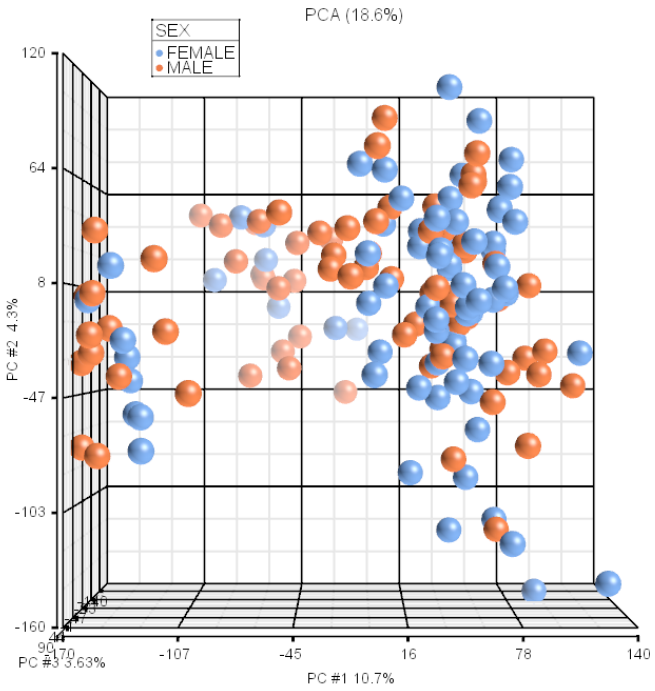


Figure 4.6. Partek Genomic Suite Histogram by Scan Date

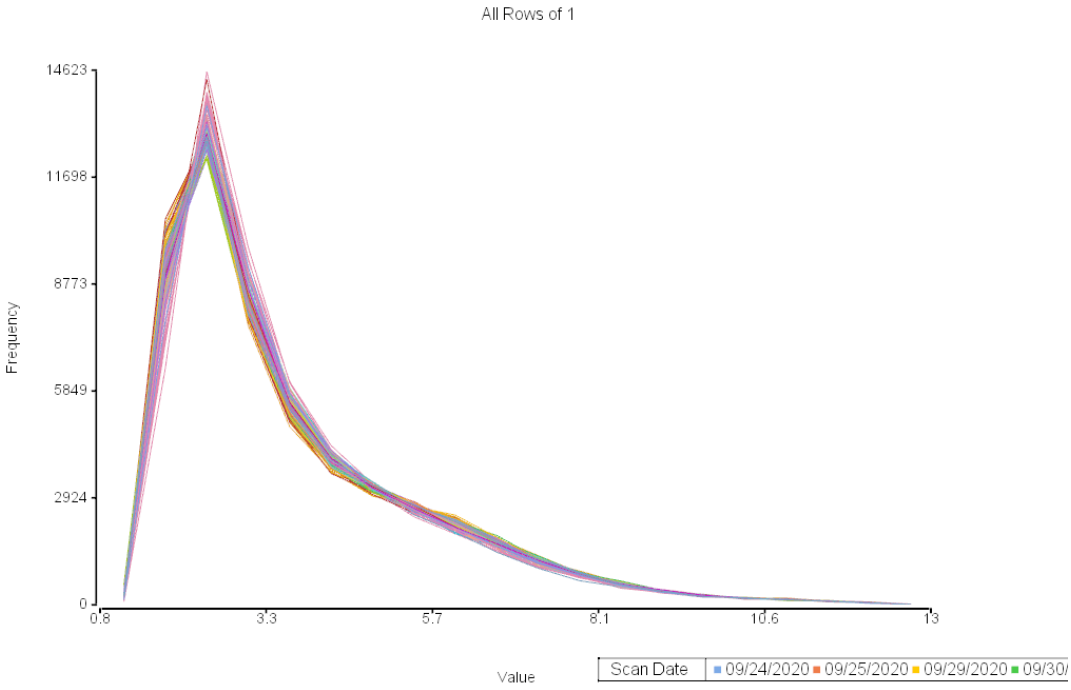


Figure 4.7. Partek Genomic Suite Histogram by Risk Group

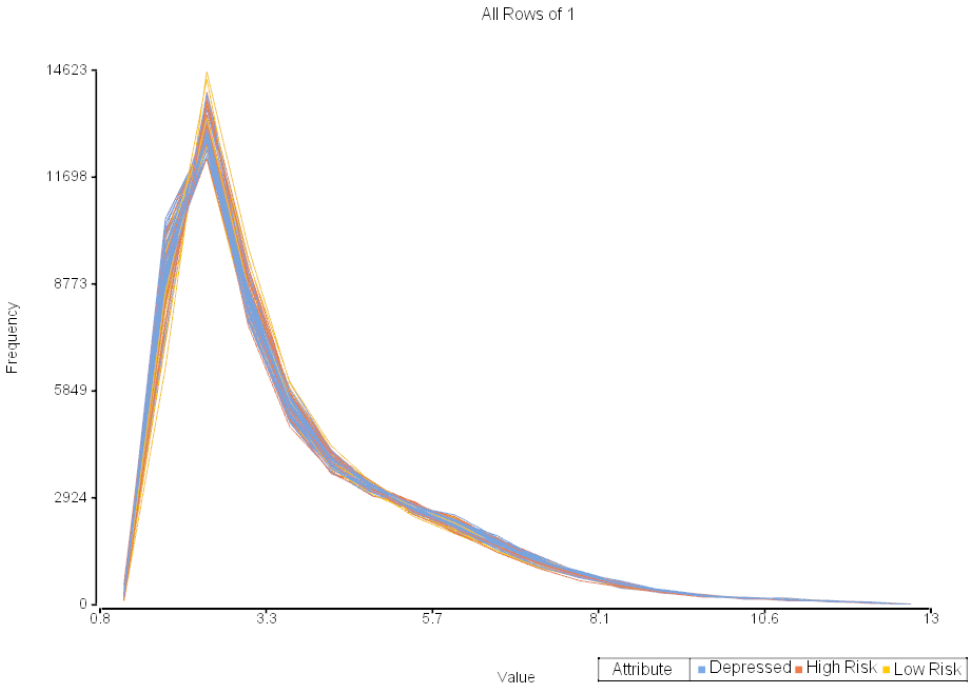


Figure 4.8. Partek Genomic Suite Sample Box & Whiskers Chart by Scan Date

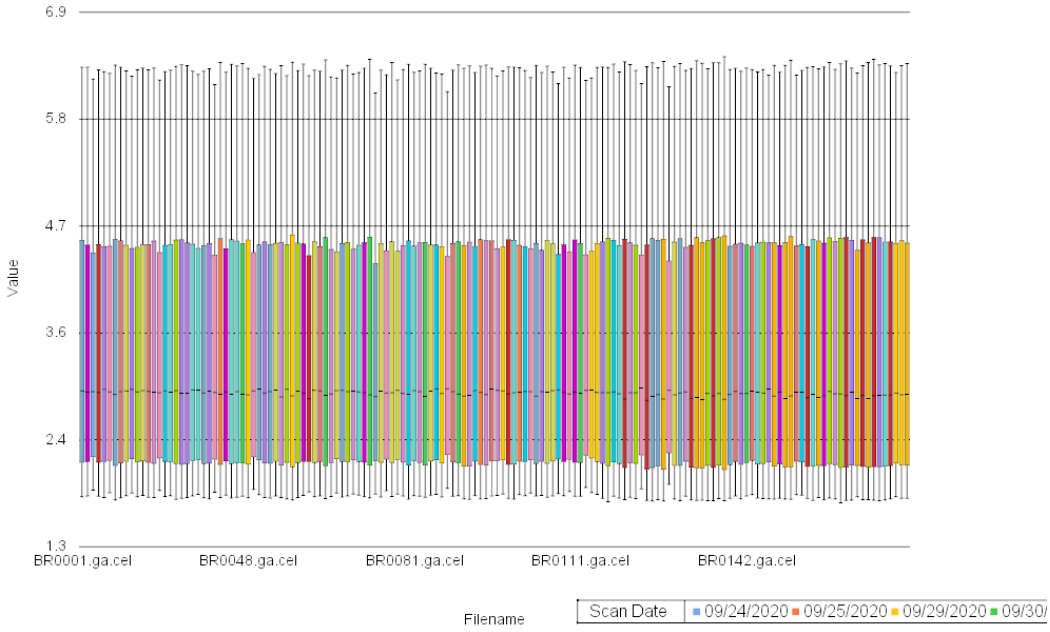
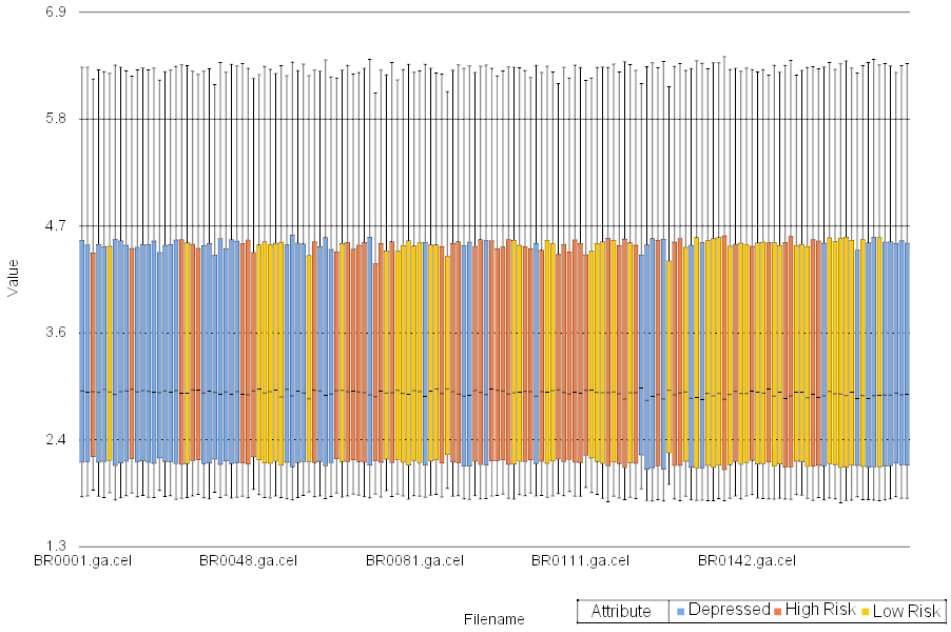


Figure 4.9. Partek Genomic Suite Sample Box & Whiskers Chart by Risk Group



4.3.3 Genes differently modulated in MDD, HR and LR adolescents

By using the Software Partek Genomic Suite, I firstly identified genes differently modulated in the comparisons among the three groups (both males and females together) specifically:

- MDD (n=50) *vs* HR adolescents (n=50)
- MDD (n=50) *vs* LR adolescents (n=50)
- HR (n=50) *vs* LR adolescents (n=50)

For each comparison, the cut offs values applied were FC ± |1.2|, p-value < 0.05 and a (FDR) cut-off of 0.05 (q-value), and the following results refer to the gene lists obtained once applied such cut-offs.

4.3.3.1 Genes differently modulated in MDD vs HR adolescents

In the comparison MDD *vs* HR, 79 genes were found differently modulated in MDD adolescents compared with HR adolescents. Among the 79 genes, 71 genes (89.9%) were up-regulated, whereas 8 (10.1%) genes were down-regulated in MDD compared with HR. Among these DEGs identified, no genes survived the FDR correction. Table 1D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 79 genes.

4.3.3.2 Genes differently modulated in MDD vs LR adolescents

In the comparison MDD *vs* LR, 23 genes were found differently modulated in MDD adolescents compared with LR adolescents. Among the 23 genes, 14 genes (60.8%) were up-regulated, whereas 9 (39.2%) genes were down-regulated in MDD compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 2D

in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 23 genes.

4.3.3.3 Genes differently modulated in HR vs LR adolescents

In the comparison HR *vs* LR, 11 genes were found differently modulated in HR adolescents compared with LR adolescents. Among the 11 genes, 2 genes (18.2%) were up-regulated, whereas 9 (81.8%) genes were down-regulated in HR compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 3D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 11 genes.

4.3.4 Genes differently modulated accordingly to biological sex: males

Raw data were analysed separately for biological sex to identify genes differently expressed by using Partek Genomic Suite. I obtained gene lists for males and females separately, and I will now report the genes differently expressed in males for the same comparison of the previous paragraph. Specifically,

- Males MDD (n=25) *vs* males HR adolescents (n=25)
- Males MDD (n=25) *vs* males LR adolescents (n=25)
- Males HR (n=25) *vs* males LR adolescents (n=25)

For each comparison, the cut offs values applied were the same of the previous analysis FC ± |1.2|, p-value < 0.05 and a (FDR) cut-off of 0.05 (q-value), and the following results refer to the gene lists obtained applied such cut-offs.

4.3.4.1 Genes differently modulated in males MDD vs males HR adolescents

In the comparison males MDD *vs* males HR, 592 genes were found differently modulated in MDD males compared with HR males. Among the 592 genes, 535 genes (90.4%) were up-regulated, whereas 57 (9.6%) genes were down-regulated in MDD compared with HR. Among these DEGs identified, no genes survived the FDR correction. Table 4D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 592 genes.

4.3.4.2 Genes differently modulated in males MDD vs males LR adolescents

In the comparison males MDD *vs* males LR, 130 genes were found differently modulated in MDD males compared with LR males. Among the 130 genes, 104 genes (80%) were up-regulated, whereas 26 (20%) genes were down-regulated in MDD

compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 5D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 130 genes.

4.3.4.3 Genes differently modulated in males HR vs males LR adolescents

In the comparison males HR *vs* males LR, 23 genes were found differently modulated in HR males compared with LR males. Among the 23 genes, 6 genes (26%) were up-regulated, whereas 17 (74%) genes were down-regulated in HR compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 6D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 23 genes.

4.3.5 Genes differently modulated accordingly to biological sex: females

Similarly to what have been explained in the paragraph 4.3.4 for males, the lists of genes differently modulated between the three groups were obtained also for females only. I will now report the genes differently expressed in females for the same comparison. Specifically,

- Females MDD (n=25) *vs* females HR adolescents (n=25)
- Females MDD (n=25) *vs* females LR adolescents (n=25)
- Females HR (n=25) *vs* females LR adolescents (n=25)

For each comparison, the cut offs values applied were the same of the previous analysis, FC ± |1.2|, p-value < 0.05 and a (FDR) cut-off of 0.05 (q-value), and the following results refer to the gene lists obtained applied such cut-offs.

4.3.5.1 Genes differently modulated in females MDD vs females HR adolescents

In the comparison females MDD *vs* females HR 42 genes were found differently modulated in MDD females compared with HR females. Among the 42 genes, 30 genes (71.4%) were up-regulated, whereas 12 (28.6%) genes were down-regulated in MDD compared with HR. Among these DEGs identified, no genes survived the FDR correction. Table 7D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 42 genes.

4.3.5.2 Genes differently modulated in females MDD vs females LR adolescents

In the comparison females MDD *vs* females LR, 67 genes were found differently modulated in MDD females compared with LR females. Among the 67 genes, 46 genes (68.6%) were up-regulated, whereas 21 (31.4%) genes were down-regulated in MDD

compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 8D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 67 genes.

4.3.5.3 Genes differently modulated in females HR vs females LR adolescents

In the comparison females HR *vs* females LR, 43 genes were found differently modulated in HR females compared with LR females. Among the 43 genes, 8 genes (18.6%) were up-regulated, whereas 35 (81.4%) genes were down-regulated in HR compared with LR. Among these DEGs identified, no genes survived the FDR correction. Table 9D in Appendix D represents the ID, Gene Assignment, p-value, and Fold-Change of the 43 genes.

4.3.6 Biological Pathways differently modulated in MDD, HR and LR

The Pathways Analysis was performed by using Ingenuity Pathways Analysis Software, which allows to obtain a list of pathways associated to an input list of genes. In this study, I uploaded the list of genes described in the previous paragraphs and reported from table 1D to 9D in Appendix D. I will now describe the pathways associated with genes differently expressed in each comparison MDD, HR and LR.

4.3.6.1 Biological Pathways differently modulated in MDD vs HR adolescents

The pathways analysis was performed on the 79 genes differently expressed in the comparison MDD *vs* HR group (for the list of genes, see table 1D in Appendix D). From the analysis, 33 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.3 (see table 1F in Appendix F for the complete list of pathways). Two pathways presented a positive z-score > 2, indicating that were up-regulated in the MDD group compared with HR: 1) Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 2.236); 2) Production of Nitric Oxide and Reactive Oxygen Species in Macrophages (z-score 2).

Moreover, many of the 33 pathways were associated with inflammation and immune system activation, such as Interferon Signaling, Complement System, IL-15 Production, Natural Killer Cell Signaling and Crosstalk Between Dendritic Cells and Natural Killer Cells. However, for none of these pathways a z-score was identified, indicating no information about their activated or inactivated status.

4.3.6.2 Biological Pathways differently modulated in MDD vs LR adolescents

The pathways analysis was performed on the 23 genes differently expressed in the comparison MDD *vs* LR group (for the list of genes, see Table 2D in Appendix D). From the analysis, 9 pathways were shown to be significantly modulated (p-value < 0.05), however no pathways were enriched for more than three genes (see table 2F in Appendix F for the complete list of pathways). The z-score was not detectable for any of the pathways. However, many of the 9 pathways were associated with inflammation and immune system activation, such as Acute Phase Response Signalling, Inflammasome Pathway, Interferon Signalling and Complement System.

4.3.6.3 Biological Pathways differently modulated in HR vs LR adolescents

The pathways analysis was performed on the 11 genes differently expressed in the comparison HR *vs* LR group (for the list of genes, see Table 3D in Appendix D). From the analysis, 11 pathways were shown to be significantly modulated (p-value < 0.05); however, no pathways were enriched for more than three genes (see table 3F in Appendix F for the complete list of pathways).

The z-score was not detectable for any of the pathways and the only gene associated with these pathways was SMOX, which was common among all the pathways.

Table 4.3. Pathways differently modulated in MDD vs HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Caveolar-mediated Endocytosis Signaling	< 0.001	0.07		COPE,FLNA,FLOT2,ITGA2B,ITGAM
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.06	2.236	IFIT2,MX1,OAS2,OAS3,STAT2
LPS/IL-1 Mediated Inhibition of RXR Function	< 0.001	0.03	0	ACSL1,CPT1A,IL18RAP,NR1H2,SMOX,TNFRSF1B
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.003	0.02	2	CYBA,JAK3,MAP3K11,TNFRSF1B
PD-1, PD-L1 cancer immunotherapy pathway	0.004	0.03		CSK,JAK3,TNFRSF1B
Paxillin Signaling	0.005	0.03		CSK,ITGA2B,ITGAM
LXR/RXR Activation	0.007	0.03		IL18RAP,NR1H2,TNFRSF1B
IL-15 Production	0.007	0.03		CSK,JAK3,MAP3K11
STAT3 Pathway	0.011	0.02		CSF2RB,IL18RAP,MAP3K11
Granulocyte Adhesion and Diapedesis	0.015	0.02		IL18RAP,ITGAM,TNFRSF1B
Acute Phase Response Signaling	0.022	0.02		C4BPA,HP,TNFRSF1B
PI3K/AKT Signaling	0.023	0.02		CSF2RB,IL18RAP,JAK3
Natural Killer Cell Signaling	0.026	0.02		IL18RAP,JAK3,MAP3K11
Integrin Signaling	0.031	0.01		ITGA2B,ITGAM,MAP3K11
Osteoarthritis Pathway	0.034	0.01		IL18RAP,NOTCH1,TNFRSF1B
Sperm Motility	0.035	0.01		CSK,JAK3,MAP3K11

4.3.7 Biological Pathways differently modulated accordingly to biological sex: males

The pathways analysis was performed also on the list of genes obtained by analysing the comparison in males and females separately. In the following section I will describe the pathways associated with genes differently expressed in males for each comparison MDD, HR and LR.

4.3.7.1 Biological Pathways differently modulated in males MDD vs males HR adolescents

The pathways analysis was performed on the 592 genes differently expressed in the comparison males MDD *vs* males HR group (for the list of genes, see table 4D in Appendix D). From the analysis, 112 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.4 (see table 4F in Appendix F for the complete list of pathways). 50 pathways presented a positive z-score > 2, indicating that were up-regulated in the MDD males, whereas 1 pathway presented a negative z-score < -2, indicating its down-regulation in MDD males compared with HR.

Many of the pathways with a z-score > 2 were associated with inflammation and immune system activation, for example: 1) Leukocyte Extravasation Signaling (z-score 2.357); 2) Th2 Pathway (z-score 2.714); 3) IL-8 Signaling (z-score 3.606); 4) Natural Killer Cell Signaling (z-score 3.742); 5) Th1 Pathway (z-score 3,); 6) IL-3 Signaling (z-score 2.121); 7) IL-9 Signaling (z-score 2); 8) IL-7 Signaling Pathway (z-score 2); 9) IL-15 Signaling (z-score 2.449). The only pathways with a negative z-score < -2 was the RhpGDI Signalling (z-score -3.162).

Moreover, many other pathways among the 112 were associated with inflammation and immune system activation, showing a general trend of pro-inflammatory status in depressed males compared with HR males.

4.3.7.2 Biological Pathways differently modulated in males MDD vs males LR adolescents

The pathways analysis was performed on the 130 genes differently expressed in the comparison males MDD *vs* males LR group (for the list of genes, see table 5D in Appendix D). From the analysis, 28 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.5 (see table 5F in Appendix F for the complete list of pathways). The z-score was detected for 6 pathways, and 5 of them reported a positive z-score > 2: 1) TREM1 Signaling (z-score 2); 2) Dendritic Cell Maturation (z-score 2); 3) Estrogen Receptor Signaling (z-score 2); 4) Insulin Secretion Signaling Pathway (z-score 2); 5) Systemic Lupus Erythematosus in B Cell Signaling Pathway (z-score 2). Moreover, although no z-score was detected, many other pathways were related to inflammation and stress response, such as IL-7 Signaling Pathway, B Cell Receptor Signaling, Interferon Signaling, and Glucocorticoid Receptor Signaling.

4.3.7.3 Biological Pathways differently modulated in males HR vs males LR adolescents

The pathways analysis was performed on the 23 genes differently expressed in the comparison males HR *vs* males LR group (for the list of genes, see table 6D in Appendix D). From the analysis, 3 pathways were shown to be significantly modulated (p-value < 0.05), however no pathways were enriched with more than three genes (see table

6F in Appendix F for the complete list of pathways). The z-score was not detectable for any of the pathways.

Table 4.4. Pathways males MDD vs males HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Integrin Signaling	< 0.001	0.1	3.638	ARPC1B,ARPC4,CAPN1,GIT1,ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,MAP3K11,NEDD9,PIK3CD,PLCG2,PXN,RAPGEF1,RHOT2,TLN1,TSPAN3,VASP
Leukocyte Extravasation Signaling	< 0.001	0.1	2.357	ARHGAP1,ARHGAP4,CYBA,ICAM3,ITGAL,ITGAM,ITGB2,MMP25,MSN,NCF2,PIK3CD,PLCG2,PRKCD,PTK2B,PXN,SIPA1,VASP,VAV1
Caveolar-mediated Endocytosis Signaling	< 0.001	0.15		COPE,DNM2,FLNA,FLOT2,HLA-E,ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2
Tec Kinase Signaling	< 0.001	0.09	3.606	GNB2,ITGA5,ITGAL,ITGB2,JAK3,PIK3CD,PLCG2,PRKCD,PTK2B,RHOT2,STAT2,STAT3,STAT5B,STAT6,TYK2,VAV1
HGF Signaling	< 0.001	0.1	3	ELF4,ITGA5,ITGAL,ITGB2,MAP3K11,MAP3K3,PIK3CD,PLCG2,PRKCD,PXN,RAPGEF1,STAT3
TREM1 Signaling	< 0.001	0.13	3	CIITA,ITGA5,ITGAX,LAT2,NLRC3,NLRC5,PLCG2,STAT3,STAT5B
Phagosome Formation	< 0.001	0.1		FCAR,IGHG1,IGHG4,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,PIK3CD,PLCG2,PRKCD,RHOT2
Th1 and Th2 Activation Pathway	< 0.001	0.08		IL4R,IL6R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT3,STAT5B,STAT6,TGFB1,TYK2,VAV1
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	< 0.001	0.11	3.162	ARPC1B,ARPC4,INPP5D,PLD3,PRKCD,PTK2B,PXN,TLN1,VASP,VAV1
Th2 Pathway	< 0.001	0.09	2.714	IL4R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT5B,STAT6,TGFB1,TYK2,VAV1
IL-8 Signaling	< 0.001	0.08	3.606	CXCR1,GNB2,IKBKE,ITGAM,ITGAX,ITGB2,LASP1,LIMK1,NCF2,PIK3CD,PLD3,PRKCD,PTK2B,RHOT2,VASP
Systemic Lupus Erythematosus Signaling	< 0.001	0.07		CD22,EFTUD2,HLA-E,IGHG1,IGHG4,IL6R,INPP5D,PIK3CD,PIM2,PLCG2,PRPF6,PRPF8,RNU4ATAC,SNRNP200,SNRPA,ZMAT5
Systemic Lupus Erythematosus In B Cell Signaling Pathway	< 0.001	0.07	2.828	CARD11,CD22,IGHA1,IGHG1,IL6R,INPP5D,LILRA6,LTB,MAVS,PIK3CD,PIM2,PLCG2,PRKCD,STAT2,STAT3,TGFB1,TYK2,VAV1
Actin Cytoskeleton Signaling	< 0.001	0.07	3.464	ARPC1B,ARPC4,FGD3,FLNA,GIT1,ITGA5,ITGAL,ITGB2,LIMK1,MSN,MYH9,NCKAP1L,PIK3CD,PXN,TLN1,VAV1

HIF1α Signaling	< 0.001	0.07	2.84	BMP6,GPI,HIF1AN,HK3,HSPA1A/HSPA1B,IL6R,MKNK2,MMP25,NCF2,PIK3CD,PKM,PLCG2,PRKCD,STAT3,TGFB1
Notch Signaling	< 0.001	0.16	0.816	FURIN,MFNG,NOTCH1,NOTCH2,PSENEN,RFNG
Role of JAK1 and JAK3 in γc Cytokine Signaling	< 0.001	0.12		FES,IL4R,JAK3,PIK3CD,PTK2B,STAT3,STAT5B,STAT6
Virus Entry via Endocytic Pathways	< 0.001	0.1		AP1M1,AP2A1,DNM2,FLNA,HLA-E,ITGA5,ITGB2,PIK3CD,PLCG2,PRKCD
Paxillin Signaling	0.001	0.09	2.236	ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,PIK3CD,PTK2B,PXN,TLN1
Natural Killer Cell Signaling	0.001	0.07	3.742	HLA-E,HSPA1A/HSPA1B,ITGAL,JAK3,LIMK1,MAP3K11,MAP3K3,NCR1,PIK3CD,PLCG2,PTK2B,PXN,TYK2,VAV1
Iron homeostasis signaling pathway	0.001	0.08		ATP6AP1,ATP6V0D1,BMP6,HBD,HBZ,IL6R,JAK3,LRP1,STAT3,STAT5B,TYK2
STAT3 Pathway	0.001	0.08	2.828	BMP6,CSF2RB,CXCR1,IGF2R,IL4R,IL6R,MAP3K11,PIM1,STAT3,TGFB1,TYK2
Th1 Pathway	0.001	0.09	3	IL6R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT3,TYK2,VAV1
IL-3 Signaling	0.001	0.1	2.121	CSF2RB,INPP5D,PIK3CD,PRKCD,RAPGEF1,STAT3,STAT5B,STAT6
Signaling by Rho Family GTPases	0.001	0.06	3.606	ARHGEF18,ARHGEF2,ARPC1B,ARPC4,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,MSN,NCF2,PIK3CD,PKN1,PTK2B,RHOT2
B Cell Receptor Signaling	0.001	0.07	2.53	BCL6,CD22,IGHA1,IGHG1,IGHG4,IKBKE,INPP5D,MAP3K11,MAP3K3,PIK3CD,PLCG2,PTK2B,VAV1
Rac Signaling	0.002	0.08	2.828	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,NCF2,PIK3CD,PTK2B
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.002	0.07	3.606	CLU,CYBA,IKBKE,JAK3,MAP3K11,MAP3K3,NCF2,PIK3CD,PLCG2,PRKCD,RHOT2,TNFRSF1B,TYK2
RhoGDI Signaling	0.002	0.07	-3.162	ARHGAP1,ARHGAP4,ARHGEF18,ARHGEF2,ARPC1B,ARPC4,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MSN,RHOT2
Ephrin Receptor Signaling	0.002	0.07	2.53	ARPC1B,ARPC4,DOK1,EPHB1,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,PXN,RAPGEF1,SH2D3C,STAT3
T Helper Cell Differentiation	0.003	0.1		BCL6,IL4R,IL6R,STAT3,STAT6,TGFB1,TNFRSF1B
Acute Myeloid Leukemia Signaling	0.003	0.09	0	CSF1R,CSF2RB,CSF3R,PIK3CD,PIM1,PIM2,STAT3,STAT5B

ERK/MAPK Signaling	0.003	0.06	3.317	ELF4,ITGA5,ITGAL,ITGB2,MKNK2,PIK3CD,PLCG2,PRKCD,PTK2B,PXN,RAPGEF1,STAT3,TLN1
PI3K/AKT Signaling	0.004	0.07	1.633	CSF2RB,CXCR1,IKBKE,IL4R,IL6R,INPP5D,ITGA5,ITGAL,ITGB2,JAK3,PIK3CD,TYK2
Phospholipase C Signaling	0.004	0.06	3	ADCY7,ARHGEF18,ARHGEF2,GNB2,HDAC1,IGHG1,IGHG4,ITGA5,ITGAL,ITGB2,ITPR3,PLCG2,PLD3,PRKCD,RHOT2
Role of JAK family kinases in IL-6-type Cytokine Signaling	0.004	0.16		IL6R,STAT3,STAT5B,TYK2
Cdc42 Signaling	0.004	0.07	2.646	ARPC1B,ARPC4,FGD3,HLA-E,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,VAV1
Macropinocytosis Signaling	0.004	0.09	2.449	ANKFY1,CSF1R,ITGA5,ITGB2,PIK3CD,PLCG2,PRKCD
Phagosome Maturation	0.005	0.07		ATP6AP1,ATP6V0D1,CTSD,DYNC1H1,GPAA1,HLA-E,NCF2,TUBB1,VPS18,VPS39
Actin Nucleation by ARP-WASP Complex	0.005	0.09	2.236	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,RHOT2,VASP
Reelin Signaling in Neurons	0.005	0.07		ARHGEF2,ARPC1B,ARPC4,ITGA5,LIMK1,MAP3K11,MAPK8IP3,PIK3CD,RAPGEF1
JAK/Stat Signaling	0.006	0.09	2.646	JAK3,PIK3CD,STAT2,STAT3,STAT5B,STAT6,TYK2
SPINK1 General Cancer Pathway	0.006	0.1	2.449	IL6R,JAK3,MT1F,PIK3CD,STAT3,TYK2
Primary Immunodeficiency Signaling	0.006	0.11		CIITA,IGHA1,IGHG1,IGHG4,JAK3
FAK Signaling	0.007	0.08		CAPN1,ITGA5,ITGAL,ITGB2,PIK3CD,PLCG2,PXN,TLN1
Cardiac Hypertrophy Signaling (Enhanced)	0.007	0.05	4.025	ADCY7,ATP2A3,CSF2RB,CXCR1,HDAC1,IKBKE,IL4R,IL6R,ITGA5,ITGAL,ITGB2,ITPR3,LTB,MAP3K11,MAP3K3,MKNK2,PIK3CD,PKN1,PLCG2,PRKCD,STAT3,TGFB1,TNFRSF1B
PAK Signaling	0.008	0.08	1.633	GIT1,ITGA5,ITGAL,ITGB2,LIMK1,PIK3CD,PTK2B,PXN
Gαq Signaling	0.008	0.07	2.333	GNB2,GRK2,IKBKE,ITPR3,PIK3CD,PLCG2,PLD3,PRKCD,PTK2B,RHOT2
Mitochondrial L-carnitine Shuttle Pathway	0.01	0.18		ACSL1,CPT1A,SLC27A3
Semaphorin Neuronal Repulsive Signaling Pathway	0.01	0.07	-0.333	FES,ITGA5,ITGAL,ITGB2,LIMK1,PIK3CD,PLCG2,SEMA4D,STK11
Crosstalk between Dendritic Cells and Natural Killer Cells	0.01	0.08	1.633	CSF2RB,HLA-E,ICAM3,ITGAL,LTB,TLN1,TNFRSF1B
Molecular Mechanisms of Cancer	0.011	0.05		ADCY7,ARHGEF18,ARHGEF2,BAK1,BMP6,CDK9,ITGA5,ITGAL,ITGB2,JAK3,LRP1,NOTCH1,PIK3CD,PRKCD,PSENEN,RAPGEF1,RHOT2,TGFB1,TYK2

IL-9 Signaling	0.011	0.12	2	JAK3,PIK3CD,STAT3,STAT5B
Huntington's Disease Signaling	0.012	0.06		CAPN1,CTSD,DCTN1,DNM2,GNB2,GPAA1,HDAC1,HSPA1A/HSPA1B,PIK3CD,POLR2E,POLR2J,POLR2J2/POLR2J3,PRKCD
Insulin Secretion Signaling Pathway	0.013	0.05	3.606	ADCY7,FURIN,GPAA1,ITPR3,JAK3,PIK3CD,PLCG2,PRKCD,STAT2,STAT3,STAT5B,STAT6,TYK2
Purine Nucleotides Degradation II (Aerobic)	0.014	0.16		ADA2,ADAT3,IMPDH1
Regulation of Cellular Mechanics by Calpain Protease	0.014	0.08		CAPN1,ITGA5,ITGAL,ITGB2,PXN,TLN1
IL-7 Signaling Pathway	0.014	0.08	2	BAK1,BCL6,IGHG1,JAK3,PIK3CD,STAT5B
Mitochondrial Dysfunction	0.014	0.06		ATP5PD,COX6A1,CPT1A,FURIN,NDUFA2,NDUFB1,NDUFB6,OGDH,PSENEN,RHOT2
Clathrin-mediated Endocytosis Signaling	0.015	0.06		AP1M1,AP2A1,ARPC1B,ARPC4,CLU,DNM2,GAK,HGS,ITGA5,ITGB2,PIK3CD
IL-15 Signaling	0.016	0.08	2.449	JAK3,PIK3CD,STAT3,STAT5B,STAT6,TYK2
Regulation of Actin-based Motility by Rho	0.016	0.07	2.236	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,LIMK1,RHOT2
Unfolded protein response	0.016	0.09		CD82,ERN1,HSPA1A/HSPA1B,OS9,VCP
Colorectal Cancer Metastasis Signaling	0.017	0.05	3	ADCY7,GNB2,GRK2,IL6R,JAK3,LRP1,MMP25,PIK3CD,PTGER4,RHOT2,STAT3,TGFB1,TYK2
T Cell Exhaustion Signaling Pathway	0.017	0.06	1	BCL6,HLA-E,IL6R,JAK3,PIK3CD,PLCG2,STAT2,STAT3,TGFB1,TYK2
RhoA Signaling	0.017	0.07	1.414	ARHGAP1,ARHGAP4,ARPC1B,ARPC4,LIMK1,MSN,PKN1,PTK2B
Semaphorin Signaling in Neurons	0.019	0.09		ARHGAP1,FES,LIMK1,RHOT2,SEMA4D
FLT3 Signaling in Hematopoietic Progenitor Cells	0.02	0.08	1.633	INPP5D,PIK3CD,STAT2,STAT3,STAT5B,STAT6
Neuregulin Signaling	0.021	0.07	1	ITGA5,ITGAL,ITGB2,PLCG2,PRKCD,RNF41,STAT5B
PD-1, PD-L1 cancer immunotherapy pathway	0.021	0.07	-0.816	HLA-E,JAK3,PIK3CD,STAT5B,TGFB1,TNFRSF1B,TYK2
IL-4 Signaling	0.023	0.07		IL4R,INPP5D,JAK3,PIK3CD,STAT6,TYK2
Triacylglycerol Degradation	0.024	0.1		ABHD2,CES2,PLB1,PNPLA2
IL-22 Signaling	0.026	0.13		STAT3,STAT5B,TYK2
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	0.026	0.13		STAT2,STAT3,TYK2
Glycolysis I	0.026	0.13		ENO1,GPI,PKM
Pancreatic Adenocarcinoma Signaling	0.027	0.06	2.449	JAK3,NOTCH1,PIK3CD,PLD3,STAT3,TGFB1,TYK2
Thrombopoietin Signaling	0.028	0.08	2.236	PIK3CD,PLCG2,PRKCD,STAT3,STAT5B

Oncostatin M Signaling	0.028	0.09	2	JAK3,STAT3,STAT5B,TYK2
PDGF Signaling	0.029	0.07	2.449	INPP5D,JAK3,PIK3CD,PLCG2,STAT3,TYK2
PI3K Signaling in B Lymphocytes	0.029	0.06	2.121	ATF6B,IKBKE,IL4R,INPP5D,ITPR3,PIK3CD,PLCG2,VAV1
ErbB2-ErbB3 Signaling	0.029	0.08	2.236	JAK3,PIK3CD,STAT3,STAT5B,TYK2
MSP-RON Signaling In Macrophages Pathway	0.03	0.06	1.134	CIITA,IKBKE,ITGAM,ITGB2,PIK3CD,SBNO2,STAT3
Sperm Motility	0.03	0.05	2.449	CSF1R,EPHB1,FES,ITPR3,JAK3,MAP3K11,PLB1,PLCG2,PRKCD,PTK2B,TYK2
IL-17A Signaling in Airway Cells	0.031	0.08	2.236	IKBKE,JAK3,PIK3CD,STAT3,TYK2
Pyridoxal 5'-phosphate Salvage Pathway	0.031	0.08	2	GRK6,LIMK1,PIM1,PKN1,PRKCD
Remodeling of Epithelial Adherens Junctions	0.032	0.08		ARPC1B,ARPC4,DNM2,HGS,TUBB1
Erythropoietin Signaling Pathway	0.034	0.05	0.333	CSF2RB,HBD,HBZ,ITPR3,LTB,PIK3CD,PRKCD,STAT5B,TGFB1
Axonal Guidance Signaling	0.035	0.04		ARPC1B,ARPC4,BMP6,EPHB1,FES,GIT1,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MMP25,NTNG2,PIK3CD,PLCG2,PRKCD,PXN,SEMA4D,TUBB1,VASP
FAT10 Cancer Signaling Pathway	0.035	0.09	2	IKBKE,STAT3,TGFB1,TNFRSF1B
PFKFB4 Signaling Pathway	0.035	0.09	1	GPI,HK3,TGFB1,TKT
fMLP Signaling in Neutrophils	0.036	0.06	2.449	ARPC1B,ARPC4,GNB2,ITPR3,NCF2,PIK3CD,PRKCD
Tight Junction Signaling	0.037	0.05		ARHGEF2,CPSF1,F2RL2,GPAA1,MYH9,SYMPK,TGFB1,TNFRSF1B,VASP
CD28 Signaling in T Helper Cells	0.038	0.06	2.646	ARPC1B,ARPC4,CARD11,IKBKE,ITPR3,PIK3CD,VAV1
Renin-Angiotensin Signaling	0.039	0.06	2.236	ADCY7,ITPR3,PIK3CD,PLCG2,PRKCD,PTK2B,STAT3
Synaptogenesis Signaling Pathway	0.04	0.05	3.606	ADCY7,AP2A1,ARPC1B,ARPC4,EPHB1,GPAA1,LIMK1,LRP1,PIK3CD,PLCG2,PRKCD,RAPGEF1,SGTA,TLN1
Spliceosomal Cycle	0.04	0.08	2	EFTUD2,SNRNP200,U2AF2,XAB2
Stearate Biosynthesis I (Animals)	0.04	0.08	2	ACOT8,ACSL1,ELOVL1,SLC27A3
GM-CSF Signaling	0.041	0.07	2	CSF2RB,PIK3CD,PIM1,STAT3,STAT5B
IL-15 Production	0.041	0.06	2.646	CSF1R,EPHB1,FES,JAK3,MAP3K11,PTK2B,TYK2
Growth Hormone Signaling	0.043	0.07	2.236	PIK3CD,PLCG2,PRKCD,STAT3,STAT5B
Ephrin B Signaling	0.045	0.07	2	EPHB1,GNB2,LIMK1,PXN,VAV1
Assembly of RNA Polymerase II Complex	0.046	0.08	1	POLR2E,POLR2J,POLR2J2/POLR2J3,TAF6
Salvage Pathways of Pyrimidine Ribonucleotides	0.046	0.06	2.236	APOBEC3A,GRK6,LIMK1,PIM1,PKN1,PRKCD

Dendritic Cell Maturation	0.047	0.05	2.828	HLA-E,IGHG1,IGHG4,IKBKE,LTB,PIK3CD,PLCG2,STAT2,TNFRSF1B

Table 4.5. Pathways males MDD vs males LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Primary Immunodeficiency Signaling	< 0.001	0.09		IGHA1,IGHG1,IGHG4,JAK3
Phagosome Formation	< 0.001	0.04		FCAR,IGHG1,IGHG4,ITGAX,PLCG2
TREM1 Signaling	< 0.001	0.06	2	ITGAX,NLRC3,NLRC5,PLCG2
Hematopoiesis from Pluripotent Stem Cells	0.001	0.07		IGHA1,IGHG1,IGHG4
Systemic Lupus Erythematosus Signaling	0.005	0.02		IGHG1,IGHG4,PLCG2,RNU4ATAC,SNRPN
Caveolar-mediated Endocytosis Signaling	0.006	0.04		COPE,FLNA,ITGAX
IL-7 Signaling Pathway	0.006	0.04		BAK1,IGHG1,JAK3
Communication between Innate and Adaptive Immune Cells	0.01	0.03		IGHA1,IGHG1,IGHG4
Dendritic Cell Maturation	0.011	0.02	2	IGHG1,IGHG4,PLCG2,STAT2
B Cell Receptor Signaling	0.013	0.02		IGHA1,IGHG1,IGHG4,PLCG2
Natural Killer Cell Signaling	0.015	0.02	1	IL18RAP,JAK3,LILRB1,PLCG2
Estrogen Receptor Signaling	0.023	0.02	2	HSP90B1,JAK3,MMP8,NOTCH1,PLCG2
Glucocorticoid Receptor Signaling	0.027	0.01		HP,HSP90B1,IL18RAP,JAK3,MMP8,POLR2J
Insulin Secretion Signaling Pathway	0.032	0.02	2	ITPR3,JAK3,PLCG2,STAT2
Phospholipase C Signaling	0.04	0.02		IGHG1,IGHG4,ITPR3,PLCG2
eNOS Signaling	0.041	0.02		HSP90B1,ITPR3,PLCG2
Gαq Signaling	0.041	0.02		GRK2,ITPR3,PLCG2
Aldosterone Signaling in Epithelial Cells	0.043	0.02		HSP90B1,ITPR3,PLCG2
Systemic Lupus Erythematosus In B Cell Signaling Pathway	0.045	0.02	2	IGHA1,IGHG1,PLCG2,STAT2

4.3.8 Biological pathways differently modulated accordingly to biological sex: females

In this section I will describe the pathways associated with genes differently expressed in females for each comparison MDD, HR and LR.

4.3.8.1 Biological Pathways differently modulated in females MDD vs females HR adolescents

The pathways analysis was performed on the 42 genes differently expressed in the comparison females MDD *vs* females HR group (for the list of genes, see table 7D in Appendix D). From the analysis, 14 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.6 (see table 7F in Appendix F for the complete list of pathways). Only one pathway presented a positive z-score > 2, indicating that it was up-regulated in MDD females, which is Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 2).

However, although the z-score was not detected, many of the pathways significantly associated with the genes differently modulated, were associated with inflammation and immune system activation, such as Interferon signaling, IL-6 signaling, and Acute Phase Response signaling.

4.3.8.2 Biological Pathways differently modulated in females MDD vs females LR adolescents

The pathways analysis was performed on the 67 genes differently expressed in the comparison females MDD *vs* females LR group (for the list of genes, see table 8D in

Appendix D). From the analysis, 12 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.7 (see supplementary table 8F in Appendix F for the complete list of pathways). A positive z-score > 2 was detected for 2 pathways: 1) Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 2.236); 2) Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses (z-score 2). Moreover, other pathways without a z-score were associated with inflammation, such as the Interferon Signaling and the Inflammasome Pathway.

4.3.8.3 Biological Pathways differently modulated in females HR vs females LR adolescents

The pathways analysis was performed on the 43 genes differently expressed in the comparison females HR *vs* females LR group (for the list of genes see table 9D in Appendix D). From the analysis, 17 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched for more than three genes are reported in table 4.8 (see table 9F in Appendix F for the complete list of pathways). The z-score was not detectable for any of these pathways. However, no pathways associated with inflammation were detected, but many pathways were associated with monoamine metabolism, such as Melatonin degradation, Putrescine degradation, Dopamine degradation, Noradrenaline and Adrenaline degradation.

Table 4.6. Pathways females MDD vs females HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.05	2	CXCL8,IFIT2,MX1,STAT2
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.001	0.05		DHX58,IFIT2,STAT2
Systemic Lupus Erythematosus In B Cell Signaling Pathway	0.007	0.01		CXCL8,IFIT2,STAT2

Table 4.7. Pathways females MDD vs females LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.06	2.236	DDX58,EIF2AK2,IFIT2,MX1,RSAD2
Interferon Signaling	< 0.001	0.08		IFIT1,IFITM3,MX1
Role of PKR in Interferon Induction and Antiviral Response	< 0.001	0.03		CASP5,DDX58,EIF2AK2,IFIH1
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.001	0.03	2	DDX58,EIF2AK2,IFIH1,TLR2
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.001	0.05		DDX58,IFIH1,IFIT2

Table 4.8. Pathways females HR vs females LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Xenobiotic Metabolism Signaling	0.008	0.01		FMO4,MAP2K3,SMOX

4.4 RNA Sequencing

4.4.1 FASTQ QA/QC

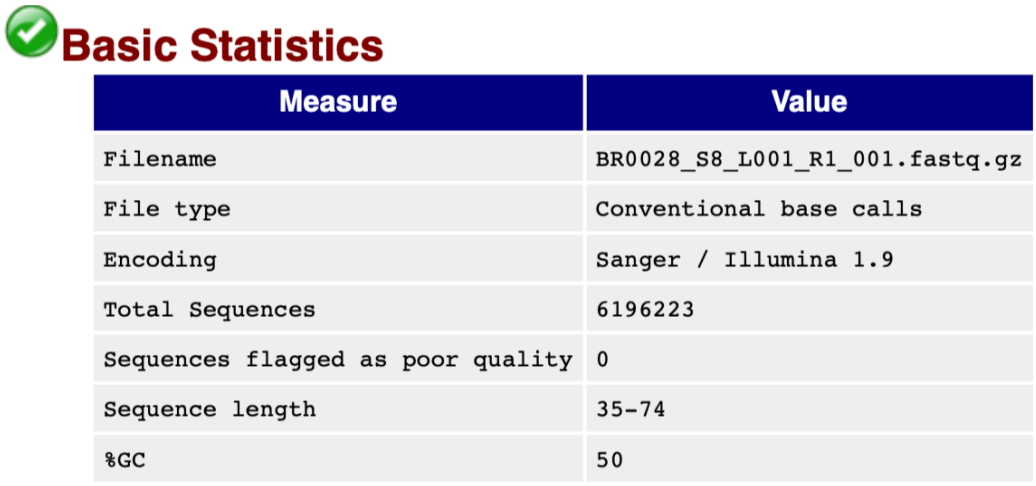
To have a general overview of the basic quality control metrics of RNA sequencing raw data, the FastQC tool was used, and the quality control analysis was performed on the entire cohort of 150 samples. For each sample, the FastQC tool provided the results and the metrics previously described in the Methods section. The profile and the metrics of all the samples were similar, showing no outliers and a general good quality of the raw data for each sample. Giving that the FastQC report accounts for several metrics and quality check, I will report the results of only one sample as an example, also given the fact that the metrics and the results of all the 150 samples were homogeneous, indicating again a good quality of the sequencing as well as a high reproducibility of the sequencing among the entire experiment.

The report provided a graphical and a list data for each model as well as a flag of "Pass" (green tick), "Warn" (yellow exclamation point) or "Fail" (red cross), which was assigned for each module. However, it is noteworthy to mention that the threshold used for flagging each module were optimized for the whole genome DNA sequencing and not for the mRNA sequencing which was performed in this doctoral thesis. Therefore, a module result that had a "Warm" or "Fail" flag did not necessarily mean that the sequence run failed, as in the following results. As an example, I will now show the FastQC results for the sample BR0028.

Basic Statistics (Figure 4.10). The sample information was reported, such as the file name and file type. A total of 6196223 sequences were reported for the sample with

0 sequences flagged as poor quality. Moreover, it was also shown the sequence length of 35-74 bp, and lastly the %GC content of 50.

Figure 4.10. FastQC Basic Statistics BR0028

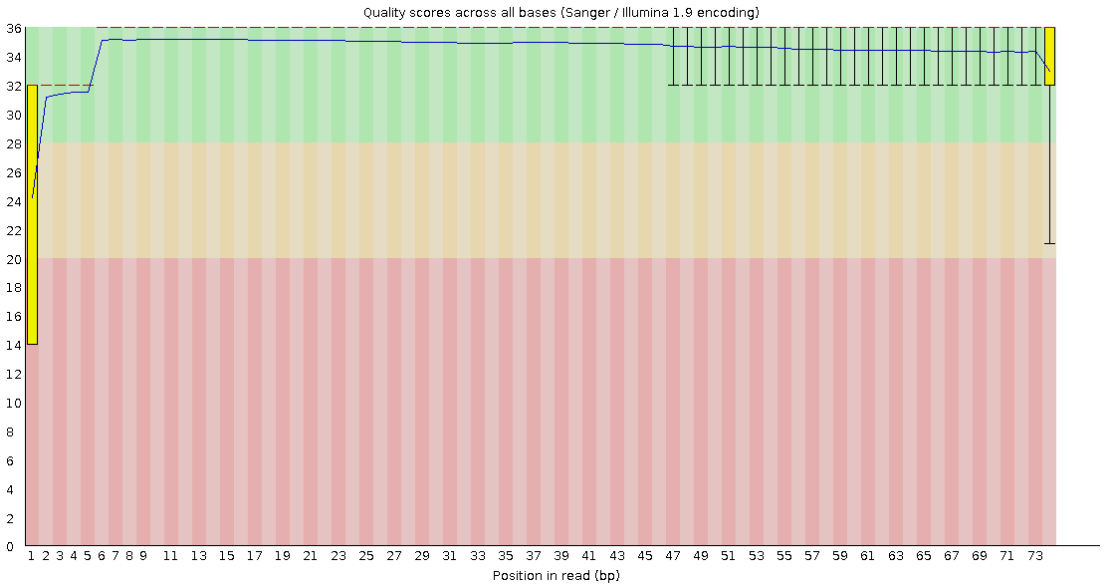


Basic Statistics

Measure	Value
Filename	BR0028_S8_L001_R1_001.fastq.gz
File type	Conventional base calls
Encoding	Sanger / Illumina 1.9
Total Sequences	6196223
Sequences flagged as poor quality	0
Sequence length	35-74
%GC	50

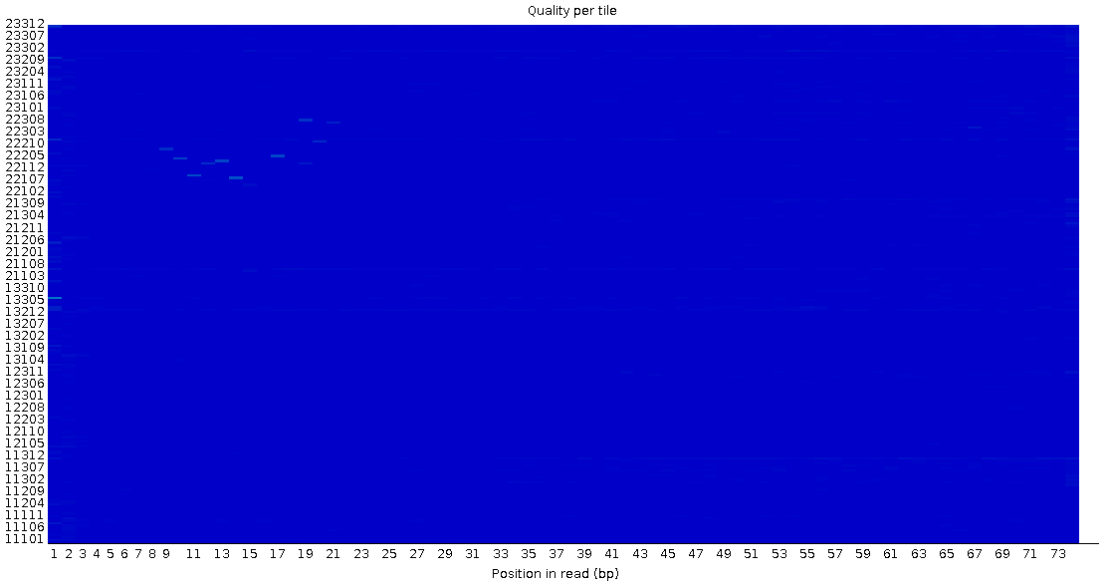
Per base Sequence Quality (Figure 4.11). This module was flag as Passed for BR0028 sample. This is a box-whisker plot showing the aggregated quality score statistics at each position along all the reads in the file. In this sample, the quality score was very high, as the quality score for each bp was within the green zone, and the higher the score the better the base call. The yellow box represents the inner-quartile range for the 25^{th} to 75^{th} percentile, whereas the upper and the lowers whiskers represent the 10^{th} and the 90^{th} percentile score. For the Illumina sequences (as the NextSeq 550) it is normal that the median quality score is lower for the first 5-7 bases and then rise; for this reason, the yellow box with a low-quality score which is shown in figure 4.11, did affect the overall quality of the run. Similarly, the average quality score is expected to steadily drop over the length of the read. The "Pass" flag indicated no degradation of quality over the duration of the run.

Figure 4.11. FastQC Per base Sequence Quality BR0028



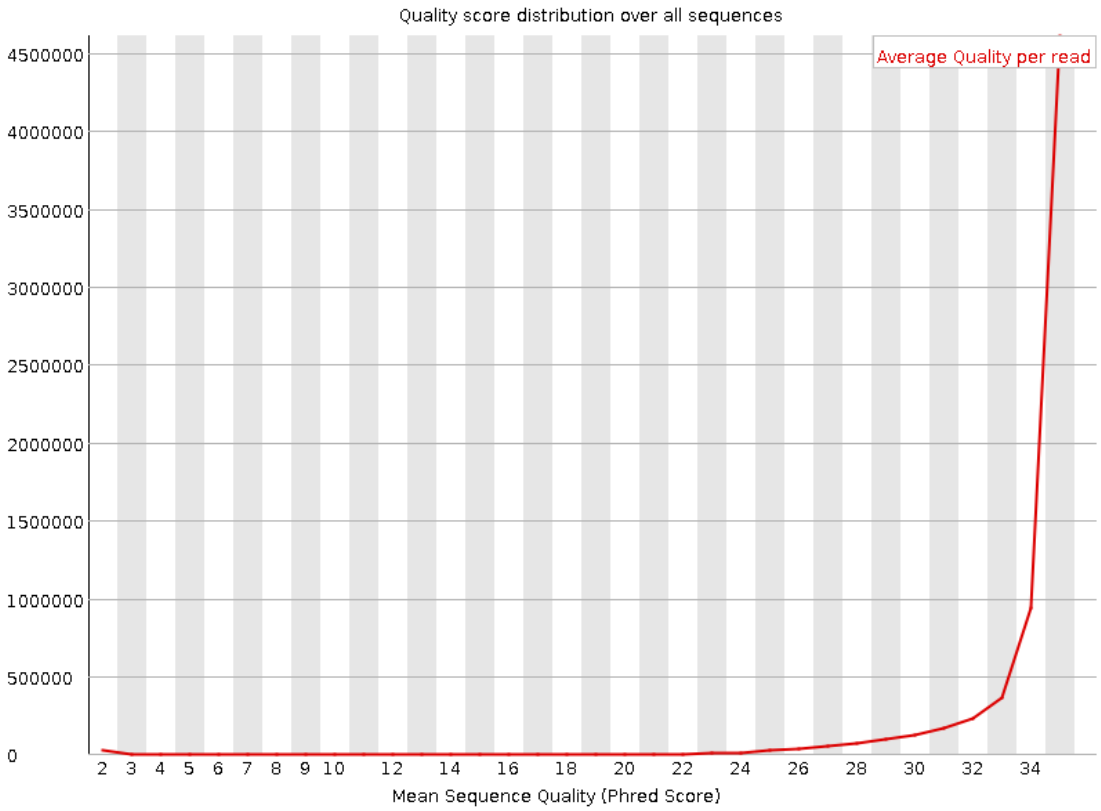
Per Tile Sequence Quality (Figure 4.12). This module was flag as Passed for BR0028 sample. This graph only appears when using an Illumina Sequencer, as for the NextSeq 550 Platform. The graph shows the quality scores from each tile across all the bases to check if there was a loss in quality associated with only one part of the flow cell. The graph's colors are on a cold to hot scale, with cold colors indicating a quality at or above the average for that base in the run, and hotter colors indicating that a tile had worse qualities than other tiles for that base. The blue color in all the plot indicated good quality of the run.

Figure 4.12. FastQC Per Tile Sequence Quality BR0028



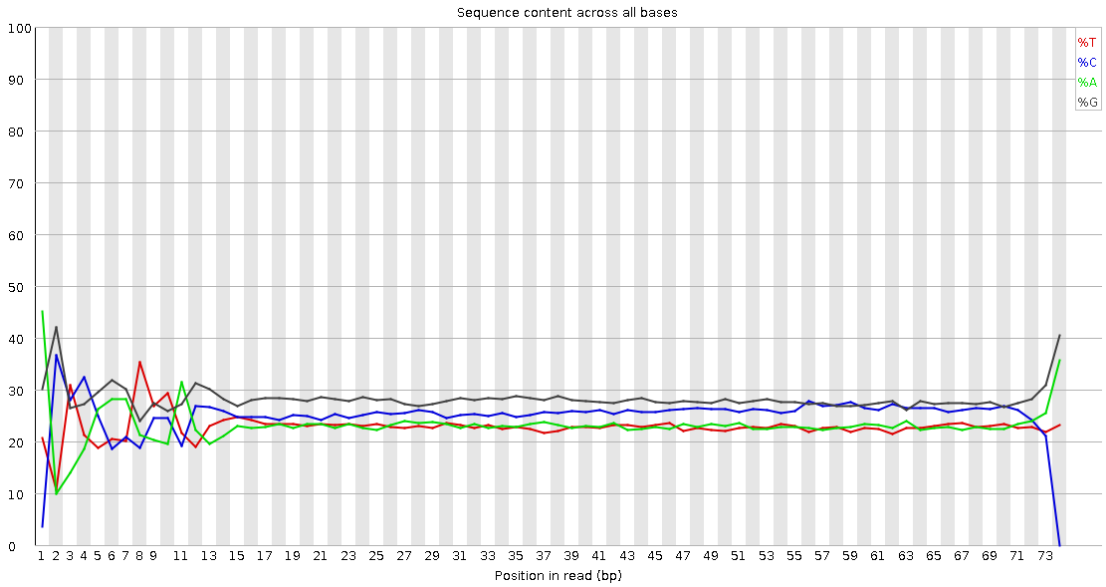
Per Sequence Quality Score (Figure 4.13). This module was flag as Passed for BR0028 sample. The distribution of average read quality was tight in the upper range of the plot, indicating good quality and no loss of quality within the run. A warning sign would have been raised if the most frequently observed mean quality was below 27, whereas failure sign if the most frequently observed mean quality was below 20.

Figure 4.13. FastQC Per Sequence Quality Score BR0028



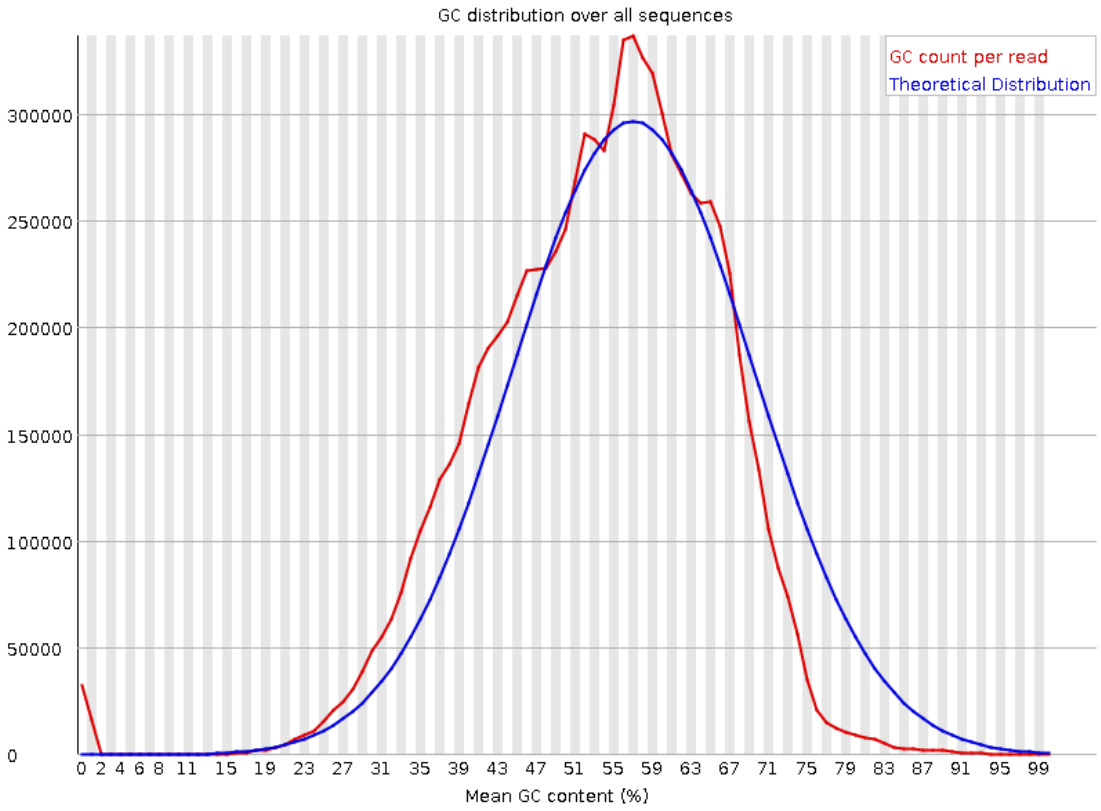
Per Base Sequence Content (Figure 4.14). This module was flag as Failed for BR0028 sample. This plot reports the percent of bases called for each of the four nucleotides at each position across all reads in the file. The failed flag did not represent a concern for this sample, as with most RNA-Seq library preparation protocols (as the one used for this analysis) there is clear non-uniform distribution of bases for the first 10-15 nucleotides, and therefore RNA-Seq data showing this non-uniform base composition are always classified as failed by FastQC for this module even though the quality of the sequencing is good.

Figure 4.14. FastQC Per Base Sequence Content BR0028



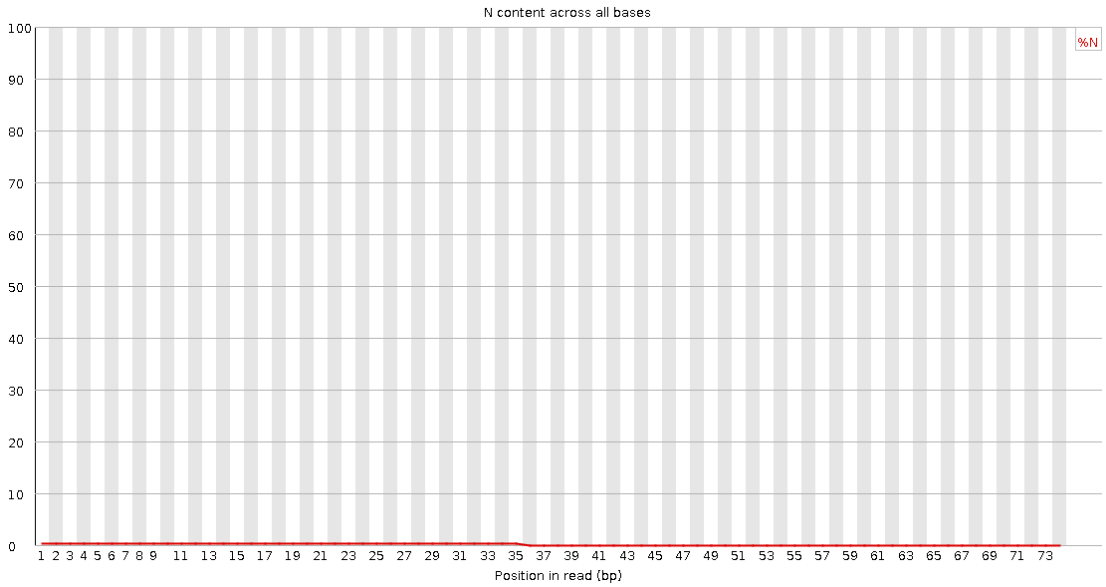
Per Sequence GC Content (Figure 4.15). This module was flag as Warn for BR0028 sample. This graph plots the number of reads versus the GC% per read. For the whole genome sequencing, the GC content of all reads is expected to form a normal distribution with the peak of the curve at the mean GC content for the organisms sequenced. However, the Warn flag shown for BR0028 graph did not affect the quality of the run, since in the RNA sequencing a greater or lesser distribution of mean GC content among transcripts can occur, and this can result in a wider or narrower plot than the theoretical distribution. Therefore, for RNA-Seq data, FastQC always assigns a Warn flag to this module.

Figure 4.15. FastQC Per Sequence GC Content BR0028



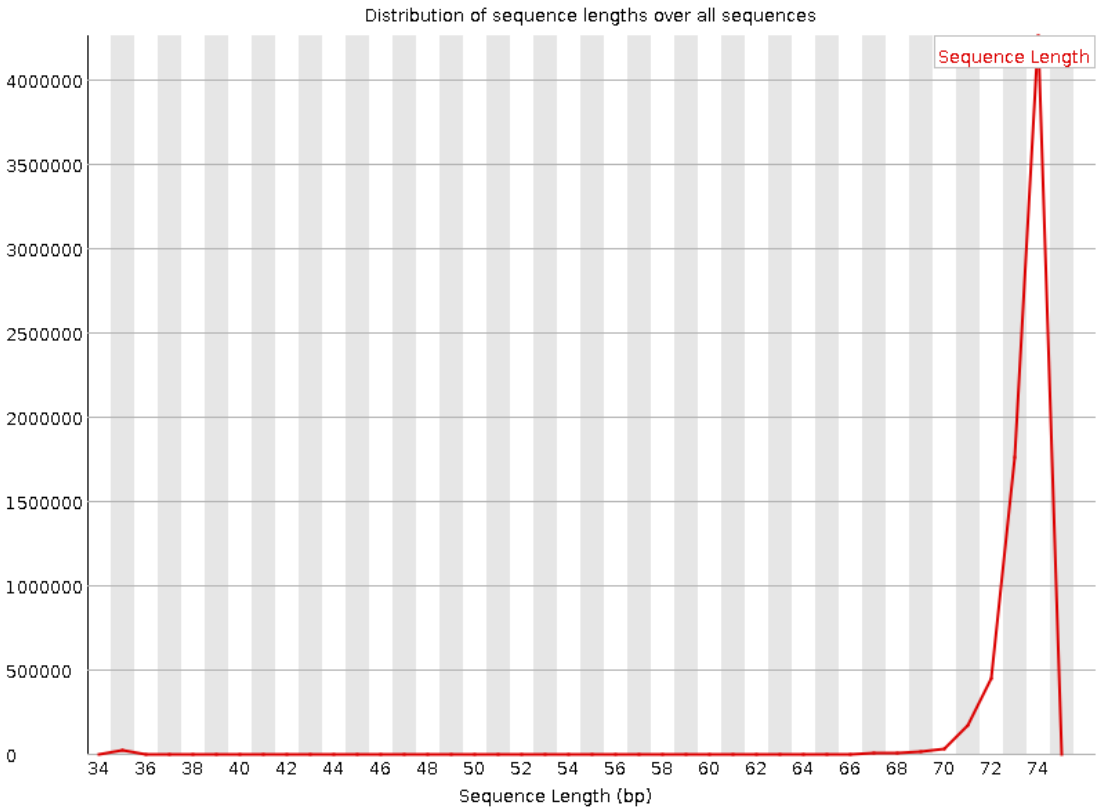
Per Base N Content (Figure 4.16). This module was flag as Pass for BR0028 sample. The absence of any point where the curve rises noticeably above zero indicate a good quality of the run. The presence of a peak indicates that an error caused the instrument to be unable to call a base at a specific position.

Figure 4.16. FastQC Per Base N Content BR0028



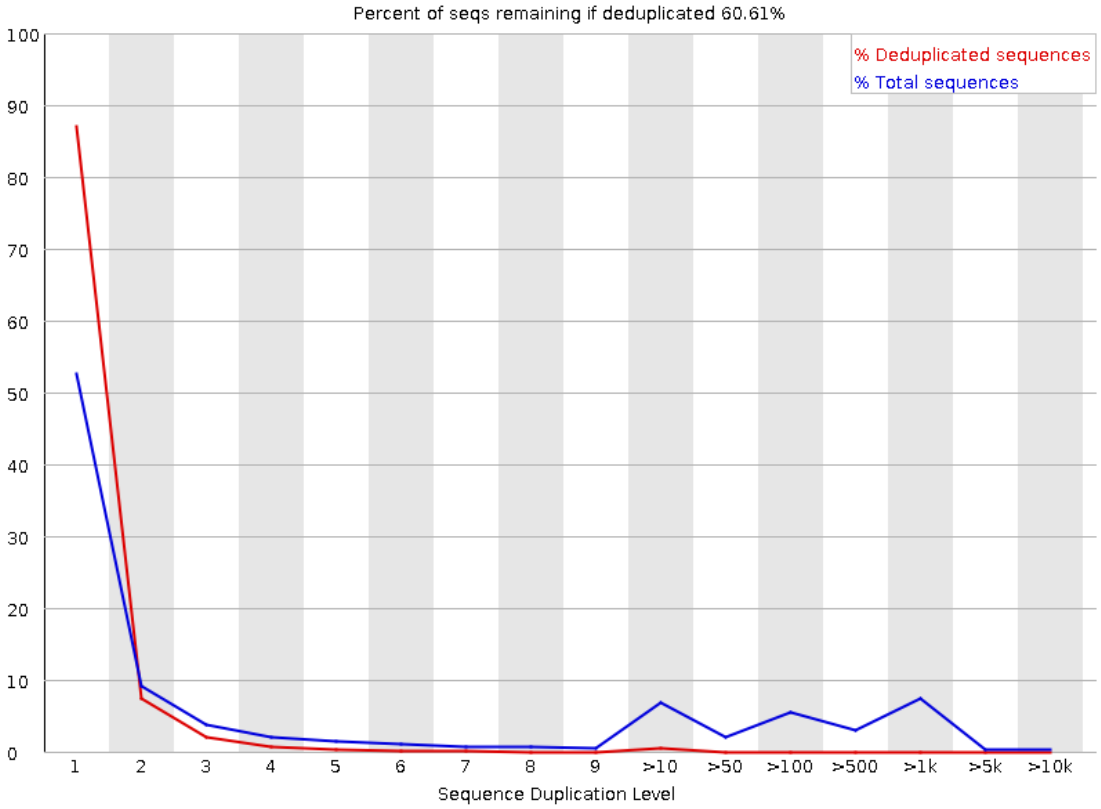
Sequence Length Distribution (Figure 4.17). This module was flag as Warn for BR0028 sample. The Warn flag was assigned due to the small peak at around 35 bp, but this did not affect the overall quality of the run.

Figure 4.17. FastQC Sequence Lenght Distribution BR0028



Sequence Duplication Levels (Figure 4.18). This module was flagged as Pass for BR0028 sample. Although it was flagged as Pass, other samples were flagged as "Warn", but this did not affect the good quality of the run because with RNA Sequencing it is normal to have very highly abundant transcripts and some lowly abundant, therefore it is expected that duplicate reads can be observed for high abundant transcripts.

Figure 4.18. FastQC Sequence Duplication Levels BR0028



Overrepresented Sequences (Figure 4.19). This module was flagged as Warn for BR0028 sample. A sequence is considered overrepresented if it accounts more than 0.1% of the total reads. The Warn flag for this sample did not affect the quality of the run, since for RNA-Seq data it is possible that there may be some transcripts that are so abundant that they are labeled as overrepresented sequence. On the other hand, in DNA-Seq data no single sequence should be present at a high frequency to be listed as overrepresented.

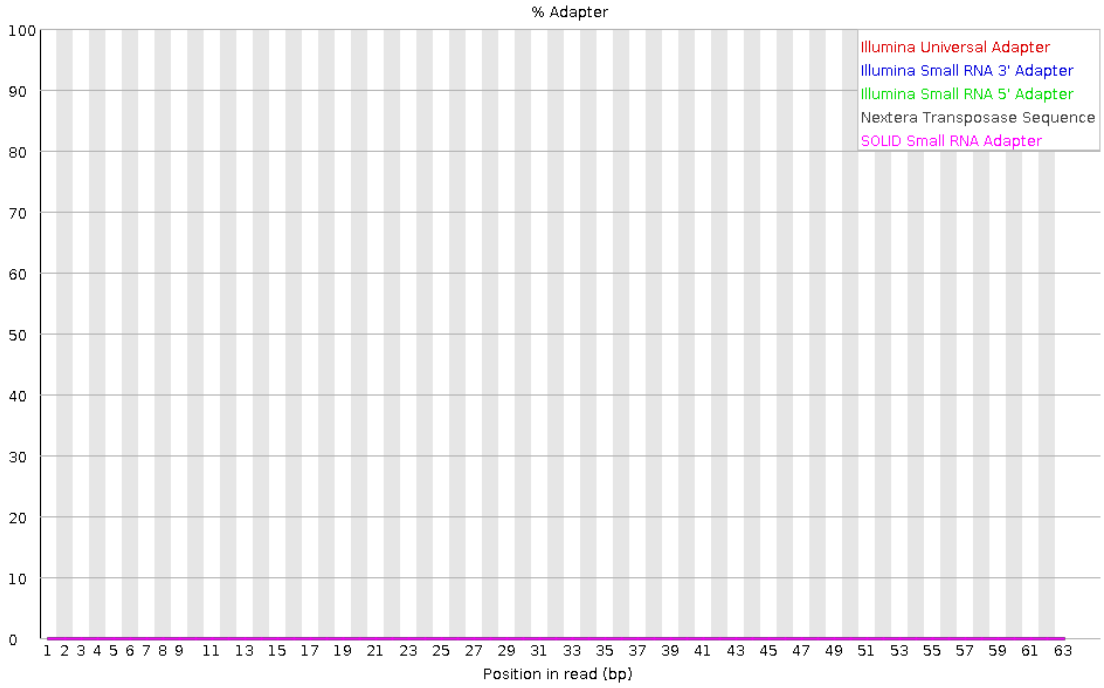
Figure 4.19. FastQC Overrepresented Sequences BR0028

Overrepresented sequences

Sequence	Count	Percentage	Possible Source
NNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNNN	37489	0.6050298706163416	No Hit

Adapter Content (Figure 4.20). This module was flag as Pass for BR0028 sample. Illumina sequence data should not have any adapter sequence; however, it is possible to have a Warn sign with RNA-Seq libraries where the distribution of library insert sizes is more varied and likely to include short inserts.

Figure 4.20. FastQC Adapter Content BR0028



4.4.2 Genes differently modulated in MDD, HR and LR adolescents

Gene differently expressed in the RNA-Seq analysis were detected by using DESeq2. I firstly identified genes differently modulated in the comparisons among the three groups (both males and females together) specifically:

- MDD (n=50) *vs* HR adolescents (n=50)
- MDD (n=50) *vs* LR adolescents (n=50)
- HR (n=50) *vs* LR adolescents (n=50)

For each comparison, to avoid a possible batch effect, the analysis was adjusted for run as covariate, considering that the 150 samples were sequenced in eleven different runs. The DEGs were expressed with their values of FC, p-value, and q-value. In the following paragraphs, I described the DEGs with applied cut offs values of FC ± |1.2|, unadjusted p-value < 0.05, and q-value < 0.05.

4.4.2.1 Genes differently modulated in MDD vs HR adolescents

In the comparison MDD *vs* HR, 313 genes were found differently modulated in MDD adolescents compared with HR adolescents. Among the 313 genes, 173 genes (55.3%) were up-regulated, whereas 140 (44.7%) genes were down-regulated in the MDD group compared with HR. Among the DEGs identified, no genes survived the FDR correction (q-value <0.05). Table 1E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 313 genes.

4.4.2.2 Genes differently modulated in MDD vs LR adolescents

In the comparison MDD *vs* LR, 461 genes were found differently modulated in MDD adolescents compared with LR adolescents. Among the 461 genes, 206 genes (44.7%)

were up-regulated, whereas 255 (55.3%) genes were down-regulated in the MDD group compared with LR. Among the DEGs identified, no genes survived the FDR correction (q-value <0.05). Table 2E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 461 genes.

4.4.2.3 Genes differently modulated in HR vs LR adolescents

In the comparison HR *vs* LR, 192 genes were found differently modulated in HR adolescents compared with LR adolescents. Among the 192 genes, 63 genes (32.8%) were up-regulated, whereas 129 (67.2%) genes were down-regulated in HR groups compared with LR. Among the DEGs identified, no genes survived the FDR correction (q-value< 0.05). Table 3E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 192 genes.

4.4.3 Genes differently modulated accordingly to biological sex: males

Similarly to the approach adopted for DEGs from microarray analysis, the DeSeq2 biostatistical analysis were performed in males and females separately. I obtained differently gene lists for males and females separately, and I will now report the genes differently expressed in males for the following comparisons:

- Males MDD (n=25) *vs* males HR adolescents (n=25)
- Males MDD (n=25) *vs* males LR adolescents (n=25)
- Males HR (n=25) *vs* males LR adolescents (n=25)

For each comparison, to avoid a possible batch effect, the analysis was adjusted for run as covariate. The DEGs were expressed with their values of FC, p-value, and q-value. In the following sections, I described the DEGs with applied cut offs values of FC ± |1.2|, unadjusted p-value < 0.05, and q-value < 0.05.

4.4.3.1 Genes differently modulated in males MDD vs males HR adolescents

In the comparison males MDD *vs* males HR, 310 genes were found differently modulated in MDD males compared with HR males. Among the 310 genes, 151 genes (48.7%) were up-regulated, whereas 159 (51.3%) genes were down-regulated in MDD males compared with HR. Among these DEGs identified, no genes survived the FDR correction (q-value < 0.05). Table 4E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 310 genes.

4.4.3.2 Genes differently modulated in males MDD vs males LR adolescents

In the comparison males MDD *vs* males LR, 377 genes were found differently modulated in MDD males compared with LR males. Among the 377 genes, 237 genes (62.9%) were up-regulated, whereas 140 (37.1%) genes were down-regulated in MDD males compared with LR. Among the identified DEGs, 1 gene survived the FDR correction (q-value < 0.05): Cochlin (COCH, q-value 0.004). Table 5E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 377 genes.

4.4.3.3 Genes differently modulated in males HR vs males LR adolescents

In the comparison males HR *vs* males LR, 359 genes were found differently modulated in HR males compared with LR males. Among the 359 genes, 215 genes (59.9%) were up-regulated, whereas 144 (40.1%) genes were down-regulated in HR males compared with LR. Among DEGs identified, no genes survived the FDR correction (q-value < 0.05). Table 6E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 359 genes.

4.4.4 Genes differently modulated accordingly to biological sex: females

The same analysis performed and described in the previous section for males, was performed for females. Thus, I will now report the genes differently expressed in females for the following comparisons:

- Females MDD (n=25) *vs* females HR adolescents (n=25)
- Females MDD (n=25) *vs* females LR adolescents (n=25)
- Females HR (n=25) *vs* females LR adolescents (n=25)

For each comparison, to avoid a possible batch effect, the analysis was adjusted for run as covariate. The DEGs were expressed with their values of FC, p-value, and q-value. In the following sections, I described the DEGs with applied cut offs values of FC ± |1.2|, unadjusted p-value < 0.05, and q-value < 0.05.

4.4.4.1 Genes differently modulated in females MDD vs females HR adolescents

In the comparison females MDD *vs* females HR, 399 genes were found differently modulated in MDD females compared with HR females. Among the 399 genes, 263 genes (65.9%) were up-regulated, whereas 136 (34.1%) genes were down-regulated in MDD females compared with HR. Among the DEGs identified, 1 gene survived the FDR correction (q-value<0.05): Chorionic Somatomammotrophin Hormone 2 (CSH2, q-value < 0.001). Table 7E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 399 genes.

4.4.4.2 Genes differently modulated in females MDD vs females LR adolescents

In the comparison females MDD *vs* females LR adolescents, 471 genes were found differently modulated in MDD females compared with LR females. Among the 471

genes, 209 genes (44.4%) were up-regulated, whereas 262 (55.6%) genes were down-regulated in MDD females compared with LR. Among DEGs identified, no genes survived the FDR correction (q-value < 0.05). Table 8E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 471 genes.

4.4.4.3 Genes differently modulated in females HR vs females LR adolescents

In the comparison females HR *vs* females LR, 504 genes were found differently modulated in HR females compared with LR females. Among the 504 genes, 197 genes (39.1%) were up-regulated, whereas 307 (60.9%) genes were down-regulated in HR females compared with LR. Among the DEGs identified, 3 genes survived the FDR correction (q-value < 0.05): Tumor Associated Calcium Transducer 2 (TACSTD2, q-value < 0.001), TBC1 Domain Family Member 3 (TBC1D3, q-value 0.005), and Sry-box Transcription Factor 5 (SOX5, q-value 0.019). Table 9E in Appendix E represents the ID, Gene Assignment, p-value, and FC of the 504 genes.

4.4.5 Biological Pathways differently modulated in MDD, HR and LR

As previously described for the microarrays section, the pathways analysis was performed by using Ingenuity Pathways Analysis Software. In this part of the study, I uploaded the list of genes described in the previous paragraphs and reported from table 1E to table 9E in the Appendix E. In this section I will describe the pathways associated with genes differently expressed in each comparison MDD, HR and LR.

4.4.5.1 Biological Pathways differently modulated in MDD vs HR adolescents

The pathways analysis was performed on the 313 genes differently expressed in the group of MDD *vs* HR (for the list of genes, see table 1E in Appendix E). From the analysis, 19 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.9 (see table 1G in Appendix G for the complete list of pathways). Four pathways presented a positive z-score > 2, indicating that were up-regulated in the MDD group: 1) Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 3.464); 2) Interferon Signaling (z-score 2.646); 3) Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses (z-score 2.449); 4) Complement System (z-score 2). On the other hand, one pathway presented a negative z-score, indicating its down-regulation in the MDD compared with HR group, which was the Coronavirus Pathogenesis Pathway (z-score -1.134). Among the 19 pathways significantly associated with the genes differently modulated in MDD *vs* HR, there were other pathways associated with inflammation and immune activation, such as the Agranulocyte Adhesion and Diapedesis, the Granulocyte Adhesion and Diapedesis, the Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza, the Role of PKR

in Interferon Induction an Antiviral Response, and the Acute Phase Response Signaling Pathway. However, for these pathways the z-score was not identified, or it was below 2, indicating no information about their activated or inactivated status in MDD group compared with HR group.

4.4.5.2 Biological Pathways differently modulated in MDD vs LR adolescents

The pathways analysis was performed on the 461 genes differently expressed in the comparison MDD *vs* LR group (for the list of genes, see table 2E in Appendix E). From the analysis, 44 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.10 (see table 2G in Appendix G for the complete list of pathways). Four pathways presented a positive z-score > 2, indicating that were up-regulated in the MDD group compared with LR: 1) Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 3.464); 2) Interferon Signaling (z-score 2.236); 3) Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses (z-score 2.646); 4) PTEN Signaling (z-score 2). On the other hand, six pathways presented a negative z-score < -2, indicating their down-regulation in MDD adolescents: 1) Calcium Signaling (z-score -2.333); 2) Glutamate Receptor Signaling (z-score -2); 3) Synaptic Long-Term Depression (z-score -2.121); 4) Neuropathic Pain Signaling in Dorsal Horm Neurons (z-score -2.646); 5) Synaptic Long Term Potentiation (z-score -2.449); 6) Regulation of Actin-based Motility by Rho (z-score -2).

Moreover, further pathways were detected with a positive or negative z-score, specifically: Neurovascular Coupling Signaling Pathways; Synaptogenesis Signaling Pathway; Activation of IRF by Cytosolic Pattern Recognition Receptors; Agrin

Interactions at Neuromuscular Junction; CREB Signaling in Neurons; SNARE Signaling Pathway; Crosstalk between Dendritic Cells and Natural Killer Cells; Amyotrophic Lateral Sclerosis Signaling; Osteoarthritis Pathway; Dilated Cardiomyopathy Signaling Pathway.

4.4.5.3 Biological Pathways differently modulated in HR vs LR adolescents

The pathways analysis was performed on the 192 genes differently expressed in the comparison HR *vs* LR group (for the list of genes, see table 3E in Appendix E). From the analysis, 41 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.11 (see table 3G in Appendix G for the complete list of pathways). Seven pathways presented a negative z-score < -2, indicating that were down-regulated in the HR group compared with LR: 1) Paxillin Signaling (z-score -2); 2) Actin Cytoskeleton Signaling (z-score -2); 3) Integrin Signaling (z-score -2.449); 4) Sperm Motility (z-score -2); 5) White Adipose Tissue Browning Pathway (z-score -2); 6) Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway (z-score -2); 7) Adrenomedullin signaling pathway (z-score -2.236). On the other hand, there were no pathways with a positive z-score > 2.

Moreover, further pathways were detected with a negative z-score > -2, specifically: Nitric Oxide Signaling in the Cardiovascular System, Pulmonary Fibrosis Idiopathic Signaling Pathway, Gαs Signaling, Neurovascular Coupling Signaling Pathway, ID1 Signaling Pathway, Osteoarthritis Pathway, Synaptic Long-Term Depression, Cardiac Hypertrophy Signaling (Enhanced).

Table 4.9. Pathways MDD vs HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.000	0.18	3.464	CCL2,CXCL10,EIF2AK2,IFIT2,IFIT3,ISG15,MX1,OAS1,OAS2,OAS3,RSAD2,TLR3
Interferon Signaling	< 0.000	0.20	2.646	IFI6,IFIT1,IFIT3,IFITM3,ISG15,MX1,OAS1
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.000	0.08	2.449	C1QA,C1QB,C1QC,EDA,EIF2AK2,IFIH1,OAS1,OAS2,OAS3,TLR3,TNFSF10
Complement System	0.001	0.13	2	C1QA,C1QB,C1QC,C4BPA
Agranulocyte Adhesion and Diapedesis	0.001	0.05		CCL2,CCL25,CCL8,CLDN12,CXCL10,GNAI1,MMP23B,MMP8,MYH11
Granulocyte Adhesion and Diapedesis	0.004	0.05		CCL2,CCL25,CCL8,CLDN12,CXCL10,GNAI1,MMP23B,MMP8
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza	0.005	0.07	0.447	CCL2,CXCL10,EIF2AK2,PLA2G2D,PLA2G4C
Estrogen-mediated S-phase Entry	0.007	0.12		CCNA1,CCNE2,CDC25A
Atherosclerosis Signaling	0.010	0.05		CCL2,COL1A2,LPL,MSR1,PLA2G2D,PLA2G4C
Activation of IRF by Cytosolic Pattern Recognition Receptors	0.011	0.07	0	DHX58,IFIH1,IFIT2,ISG15
Role of PKR in Interferon Induction and Antiviral Response	0.013	0.05		COLEC12,EIF2AK2,IFIH1,MARCO,MSR1,TLR3
Hepatic Fibrosis / Hepatic Stellate Cell Activation	0.018	0.04		BAMBI,CCL2,COL15A1,COL1A2,COL4A4,MYH11,SERPINE1
Coronavirus Pathogenesis Pathway	0.024	0.04	-1.134	CCL2,CCNE2,OAS1,OAS2,OAS3,SERPINE1,TLR3
Role of MAPK Signaling in the Pathogenesis of Influenza	0.030	0.05		CCL2,CXCL10,PLA2G2D,PLA2G4C
Oxytocin Signaling Pathway	0.032	0.03	0.378	ATP2B3,GNAI1,KCNJ8,LPL,MYH11,PLA2G2D,PLA2G4C,PPARG
Acute Phase Response Signaling	0.048	0.04		C1QA,C1QB,C1QC,C4BPA,HP,SERPINE1

Table 4.10. Pathways MDD vs LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.000	0.18	3.46	CCL2,DDX58,EIF2AK2,IFIT2,IFIT3,IL1RN,IRF7,ISG15,MX1,OAS2,OAS3,RSAD2
Calcium Signaling	< 0.000	0.08	-2.33	ACTC1,ATP2B2,CAMK2A,CASQ1,CHRNB2,GRIA1,GRIA2,GRIA3,GRIN1,MYH10,MYH11,RYR1,TP63
Glutamate Receptor Signaling	< 0.000	0.13	-2.00	GRIA1,GRIA2,GRIA3,GRIN1,GRM6,HOMER1,SLC1A2
Neurovascular Coupling Signaling Pathway	< 0.000	0.07	-1.94	GABRD,GABRR2,GAD1,GRIA1,GRIA2,GRIA3,GRIN1,KCNJ10,KCNJ2,KCNMA1,NPR1,RYR1,SLC1A2
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.000	0.08	2.65	C1QB,C1QC,DDX58,EDA,EIF2AK2,IFIH1,IRF7,OAS2,OAS3,TLR5,TNFSF10
Synaptogenesis Signaling Pathway	< 0.000	0.06	-1.81	ADCY2,AFDN,CAMK2A,EFNB2,EPHA5,EPHA7,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,RASD2,STXBP1,STXBP4,STXBP6,WASF1
Gap Junction Signaling	0.001	0.07		ACTC1,ACTG2,ADCY2,GAD1,GRIA1,GRIA2,GRIA3,HTR2B,NPR1,RASD2,TJP1,TUBB3
Interferon Signaling	0.001	0.14	2.24	IFI6,IFIT1,IFIT3,ISG15,MX1
Activation of IRF by Cytosolic Pattern Recognition Receptors	0.002	0.11	1.63	DDX58,IFIH1,IFIT2,IRF7,ISG15,ZBP1
Agrin Interactions at Neuromuscular Junction	0.002	0.10	-1.00	ACTC1,ACTG2,AGRN,ERBB4,LAMA2,RASD2
Synaptic Long Term Depression	0.003	0.06	-2.12	CRHR1,GAD1,GRIA1,GRIA2,GRIA3,GRM6,NPR1,PPP2R2C,RASD2,RYR1
Neuropathic Pain Signaling In Dorsal Horn Neurons	0.005	0.08	-2.65	CAMK2A,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,KCNQ2
CREB Signaling in Neurons	0.006	0.04	-1.96	ADCY2,ADGRA3,ADGRG6,ADGRL3,BMP6,CAMK2A,CRHR1,FFAR3,FGFR4,GPR176,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,HCAR2,HCAR3,HTR2B,NTRK3,RASD2,TACR3
Tight Junction Signaling	0.008	0.06		ACTC1,ACTG2,AFDN,MAGI2,MYH10,MYH11,NECTIN2,PPP2R2C,TJP1
SNARE Signaling Pathway	0.009	0.07	-1.89	ADCY2,CAMK2A,MYH10,MYH11,STXBP1,STXBP4,STXBP6
Sertoli Cell-Sertoli Cell Junction Signaling	0.010	0.05		ACTC1,ACTG2,AFDN,ITGA8,ITGA9,MAGI2,NECTIN2,RASD2,TJP1,TUBB3
Ephrin Receptor Signaling	0.011	0.05		EFNB2,EPHA5,EPHA7,GRIN1,ITGA8,ITGA9,PTPN13,RASD2,SDC2,VEGFC
Glutathione-mediated Detoxification	0.011	0.14		GGH,GSTA1,GSTA4

Crosstalk between Dendritic Cells and Natural Killer Cells	0.013	0.07	1	ACTC1,ACTG2,CAMK2A,CD80,NECTIN2,TNFSF10
Cellular Effects of Sildenafil (Viagra)	0.014	0.06		ACTC1,ACTG2,ADCY2,KCNQ2,MYH10,MYH11,NPR1
Synaptic Long Term Potentiation	0.017	0.06	-2.45	CAMK2A,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,RASD2
Epithelial Adherens Junction Signaling	0.018	0.06	0	AFDN,MAGI1,MAGI2,MYH10,NECTIN2,PPP2R2C,RASD2,WASF1
Adrenomedullin signaling pathway	0.021	0.05	-0.38	ADCY2,ADM,GAD1,IL1RN,KCNQ2,NPR1,PPARG,RASD2,TFAP2A
Regulation of Actin-based Motility by Rho	0.023	0.06	-2	ACTC1,ACTG2,ITGA8,ITGA9,PFN2,WASF1
Airway Pathology in Chronic Obstructive Pulmonary Disease	0.024	0.06		CCL2,EDA,ELANE,LCN2,MMP8,TNFSF10
Amyotrophic Lateral Sclerosis Signaling	0.025	0.06	-0.82	GRIA1,GRIA2,GRIA3,GRIN1,SLC1A2,VEGFC
Gustation Pathway	0.026	0.05	0	ABCC8,ADCY2,ASIC2,GABRD,GABRR2,KCNQ2,LPL,SCN4B
cAMP-mediated signaling	0.026	0.05	0	ADCY2,CAMK2A,CRHR1,FFAR3,GRM6,HCAR2,HCAR3,PDE8B,RGS4,TULP2
Osteoarthritis Pathway	0.029	0.05	0.38	CASP5,CASQ1,DCN,IL18RAP,ITGA8,ITGA9,PPARG,PRG4,SMAD1,VEGFC
Oxytocin In Spinal Neurons Signaling Pathway	0.031	0.10		ABCC8,GAD1,NPR1
Complement System	0.031	0.10		C1QB,C1QC,C4BPA
Breast Cancer Regulation by Stathmin1	0.035	0.04	0	ADGRA3,ADGRG6,ADGRL3,BMP6,CAMK2A,CRHR1,E2F7,FFAR3,GPR176,GRM6,HCAR2,HCAR3,HTR2B,PPP2R2C,RASD2,TACR3,TUBB3,VEGFC
Airway Inflammation in Asthma	0.039	0.09		CCL2,ELANE,RNASE2
Circadian Rhythm Signaling	0.041	0.04		ADCY2,CAMK2A,GAD1,GRIA1,GRIA2,GRIA3,GRIN1,NPR1,RASD2,RYR1
Axonal Guidance Signaling	0.043	0.04		ADAMDEC1,BMP6,BMP8A,EFNB2,EPHA5,EPHA7,ITGA8,ITGA9,MMP8,NTNG2,NTRK3,PFN2,RASD2,SDC2,TUBB3,VEGFC
Agranulocyte Adhesion and Diapedesis	0.044	0.05		ACTC1,ACTG2,CCL2,IL1RN,MMP8,MYH10,MYH11,XCL1
Dilated Cardiomyopathy Signaling Pathway	0.046	0.05	-0.45	ACTC1,ACTG2,ADCY2,CAMK2A,MYH10,MYH11
PTEN Signaling	0.049	0.05	2	FGFR4,ITGA8,ITGA9,MAGI1,MAGI2,NTRK3,RASD2

Table 4.11. Pathways HR vs LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Pulmonary Fibrosis Idiopathic Signaling Pathway	< 0.000	0.04	-1.265	ACTG2,AREG,CAV1,COL16A1,FGF9,FGFR4,ITGAV,MMP1,MRAS,PDGFC,PLG
Nitric Oxide Signaling in the Cardiovascular System	0.001	0.06	-1.342	CAV1,GUCY1A1,PDE1A,PDGFC,PRKG2,RYR2
Sertoli Cell-Sertoli Cell Junction Signaling	0.002	0.04		ACTG2,GUCY1A1,ITGAV,MRAS,NECTIN2,PRKG2,TUBB3
Neurovascular Coupling Signaling Pathway	0.002	0.04	-1.134	ENTPD3,GRIN3B,GRM5,GUCY1A1,KCNJ10,PRKG2,RYR2
Paxillin Signaling	0.003	0.05	-2	ACTG2,DOCK1,ITGAV,MRAS,TLN2
Actin Cytoskeleton Signaling	0.003	0.03	-2	ACTG2,DOCK1,FGF9,ITGAV,MRAS,PDGFC,TLN2
Gαs Signaling	0.005	0.04	-1	GLP1R,GUCY1A1,MRAS,PTH1R,RYR2
Cellular Effects of Sildenafil (Viagra)	0.005	0.04		ACTG2,GUCY1A1,KCNQ2,PDE1A,PRKG2
Gap Junction Signaling	0.006	0.03		ACTG2,CAV1,GUCY1A1,MRAS,PRKG2,TUBB3
Axonal Guidance Signaling	0.008	0.02		DOCK1,GLI3,ITGAV,MMP1,MRAS,NFATC4,NTRK2,PDGFC,SRGAP1,TUBB3
Integrin Signaling	0.009	0.03	-2.449	ACTG2,CAV1,DOCK1,ITGAV,MRAS,TLN2
ID1 Signaling Pathway	0.009	0.03	-1.633	CAV1,CHRFAM7A,FGFR4,MRAS,PDGFC,TFAP2A
Neuropathic Pain Signaling In Dorsal Horn Neurons	0.012	0.04	-1	GRIN3B,GRM5,KCNQ2,NTRK2
Cardiac β-adrenergic Signaling	0.015	0.03		GUCY1A1,MRAS,PDE1A,RYR2,SLC8A2
Osteoarthritis Pathway	0.015	0.03	-1	GLI3,ITGAV,ITLN1,MMP1,PDGFC,PTH1R
Bladder Cancer Signaling	0.016	0.04		FGF9,MMP1,MRAS,PDGFC
Sperm Motility	0.018	0.03	-2	FGFR4,GUCY1A1,MRAS,NTRK2,PDE1A,PRKG2
Synaptic Long Term Depression	0.020	0.03	-1.342	GRM5,GUCY1A1,MRAS,PRKG2,RYR2
Gustation Pathway	0.020	0.03	0	GLP1R,GUCY1A1,KCNQ2,SCN2A,SCN4B
Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway	0.023	0.03	-2	FGF9,FGFR4,MMP1,MRAS,PDGFC
Circadian Rhythm Signaling	0.025	0.02		GRIN3B,GUCY1A1,MRAS,NTRK2,PRKG2,RYR2
Glioma Invasiveness Signaling	0.026	0.04		ITGAV,MRAS,PLG
Adrenomedullin signaling pathway	0.026	0.03	-2.236	GUCY1A1,KCNQ2,MRAS,PRKG2,TFAP2A
Calcium Signaling	0.027	0.03	0.447	CHRFAM7A,GRIN3B,NFATC4,RYR2,SLC8A2
White Adipose Tissue Browning Pathway	0.028	0.03	-2	FGFR4,GUCY1A1,PLIN1,PRKG2

Caveolar-mediated Endocytosis Signaling	0.028	0.04		ACTG2,CAV1,ITGAV
Opioid Signaling Pathway	0.034	0.02	-0.447	GRIN3B,GUCY1A1,MRAS,PDE1A,POMC,RYR2
STAT3 Pathway	0.035	0.03		FGFR4,IL9R,MRAS,NTRK2
Semaphorin Neuronal Repulsive Signaling Pathway	0.035	0.03	0	DPYSL4,GUCY1A1,ITGAV,PRKG2
Cardiac Hypertrophy Signaling (Enhanced)	0.040	0.02	-0.378	FGF9,FGFR4,GUCY1A1,IL9R,ITGAV,MRAS,NFATC4,PDE1A,RYR2
PDGF Signaling	0.040	0.04		CAV1,MRAS,PDGFC
Regulation of Cellular Mechanics by Calpain Protease	0.044	0.04		ITGAV,MRAS,TLN2
PTEN Signaling	0.045	0.03		FGFR4,ITGAV,MRAS,NTRK2
Crosstalk between Dendritic Cells and Natural Killer Cells	0.045	0.03		ACTG2,NECTIN2,TLN2
Synaptogenesis Signaling Pathway	0.046	0.02	-0.447	GRIN3B,GRM5,GUCY1A1,MRAS,NTRK2,STXBP4
Human Embryonic Stem Cell Pluripotency	0.050	0.03		FGFR4,MRAS,NTRK2,PDGFC

4.4.6 Biological Pathways differently modulated accordingly to biological sex: males

The pathways analysis was performed also on the list of genes obtained by analysing only the comparison in males and females separately.

In this section, I will describe the pathways associated with genes differently expressed in males for each comparison MDD, HR and LR.

4.4.6.1 Biological Pathways differently modulated in males MDD vs males HR adolescents

The pathways analysis was performed on the 310 genes differently expressed in the comparison males MDD *vs* males HR group (for the list of genes, see table 4E in Appendix E). From the analysis, 10 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.12 (see table 4G in Appendix G for the complete list of pathways). Two pathways presented a positive z-score < 2, indicating a low up-regulation in MDD males compared with HR: 1) Mitotic Roles of Polo-Like Kinase (z-score 1.342); 2) Kinetochore Metaphase Signalling Pathway (z-score 0.378).

4.4.6.2 Biological Pathways differently modulated in males MDD vs males LR adolescents

The pathways analysis was performed on the 377 genes differently expressed in the comparison males MDD *vs* males LR group (for the list of genes, see table 5E in Appendix E). From the analysis, 27 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.13 (see table 5G in Appendix G for the complete list of

pathways). A positive z-score > 2 was detected for 3 pathways: 1) Estrogen-mediated S-phase Entry (z-score 2.449); 2) Cyclins and Cell Cycle Regulation (z-score 2.828); 3) p53 Signaling (z-score 2.236). On the other hand, one pathway had a negative z-score < -2, indicating that was down-regulated in MDD males compared with LR: Cell Cycle: G1/S Checkpoint Regulation (z-score -2.236).

Moreover, other pathways were identified with a non-significant positive or negative z-score, specifically, Mitotic Roles of Polo-Like Kinase; Role of CHK Proteins in Cell Cycle Checkpoint Control; Kinetochore Metaphase Signaling Pathway; Epithelial Adherens Junction Signaling; Osteoarthritis Pathway; Pulmonary Healing Signaling Pathway; Cell Cycle: G2/M DNA Damage Checkpoint Regulation; ATM Signaling; Role of BRCA1 in DNA Damage Response.

4.4.6.3 Biological Pathways differently modulated in males HR vs males LR adolescents

The pathways analysis was performed on the 359 genes differently expressed in the comparison males HR *vs* males LR group (for the list of genes, see table 6E in Appendix E). From the analysis, 8 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.14 (see table 6G in Appendix G for the complete list of pathways). One pathway was shown to have a negative z-score < -2: Oxidative Phosphorylation (z-score -2.646).

On the other hand, one pathway showed a positive z-score < 2: Leukocyte Extravasation Signaling (z-score 0.816).

Table 4.12. Pathways males MDD vs males HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Mitotic Roles of Polo-Like Kinase	< 0.000	0.12	1.342	CDC20,CDC25A,ESPL1,KIF11,PKMYT1,PLK1,PPM1J
Kinetochore Metaphase Signaling Pathway	0.001	0.07	0.378	AURKB,BUB1,CDC20,ESPL1,KNL1,PLK1,SKA3
Role of CHK Proteins in Cell Cycle Checkpoint Control	0.009	0.08		CDC25A,CLSPN,PLK1,PPM1J
Intrinsic Prothrombin Activation Pathway	0.017	0.09		COL1A2,COL5A3,KLK1
Atherosclerosis Signaling	0.035	0.04		COL1A2,COL5A3,LPL,PDGFB,PLA2G2D

Table 4.13. Pathways males MDD vs males LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Mitotic Roles of Polo-Like Kinase	< 0.000	0.17	1.890	CCNB1,CDC20,CDC25A,CDK1,ESPL1,HSP90B1,KIF11,PKMYT1,PLK1,PPP2R3A
Role of CHK Proteins in Cell Cycle Checkpoint Control	< 0.000	0.17	-1.890	BRCA1,CDC25A,CDK1,CLSPN,E2F2,E2F7,E2F8,PLK1,PPP2R3A
Kinetochore Metaphase Signaling Pathway	< 0.000	0.12	1.508	AURKB,BUB1B,CCNB1,CDC20,CDK1,CENPE,ESPL1,KNL1,MAD1L1,PLK1,ZWINT
Estrogen-mediated S-phase Entry	< 0.000	0.23	2.449	CCNA2,CDC25A,CDK1,E2F2,E2F7,E2F8
Cyclins and Cell Cycle Regulation	< 0.000	0.10	2.828	CCNA2,CCNB1,CDC25A,CDK1,E2F2,E2F7,E2F8,PPP2R3A
Epithelial Adherens Junction Signaling	0.001	0.07	-1.265	AFDN,CTNNA2,MAGI1,MAGI2,MET,MYH10,NECTIN2,NOTCH3,PPP2R3A,WASF1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	0.003	0.10	-0.447	BRCA1,CCNB1,CDK1,PKMYT1,PLK1
Cell Cycle Regulation by BTG Family Proteins	0.005	0.12		E2F2,E2F7,E2F8,PPP2R3A
DNA damage-induced 14-3-3σ Signaling	0.005	0.17		BRCA1,CCNB1,CDK1
Glutathione-mediated Detoxification	0.008	0.15		GGH,GSTA1,PTGES
ATM Signaling	0.011	0.07	0	BRCA1,CCNB1,CDC25A,CDK1,PPP2R3A,TP73
Cell Cycle: G1/S Checkpoint Regulation	0.012	0.07	-2.236	CDC25A,E2F2,E2F7,E2F8,NRG1
Osteoarthritis Pathway	0.015	0.05	0.378	CTNNA2,DCN,ELF3,FZD7,IL18RAP,ITGA8,JAG1,PPARG,PPARGC1A,VEGFC
Pulmonary Healing Signaling Pathway	0.016	0.05	1	CCNB1,CTRC,ELANE,FZD7,JAG1,MMP8,NOTCH3,PRKD1,VEGFC
Role of BRCA1 in DNA Damage Response	0.019	0.07	-0.447	BRCA1,E2F2,E2F7,E2F8,PLK1
Phospholipases	0.028	0.07		GPLD1,LPL,PLA2G12A,PLAAT2
Triacylglycerol Degradation	0.040	0.08		FAAH,LPL,PNPLA4
Inhibition of Matrix Metalloproteases	0.040	0.08		MMP8,SDC1,SDC2
Iron homeostasis signaling pathway	0.041	0.05		BMP6,BMP8A,BMP8B,HBA1/HBA2,HP,TFR2
p53 Signaling	0.044	0.05	2.236	BRCA1,PERP,PLAGL1,TP63,TP73
Basal Cell Carcinoma Signaling	0.047	0.06		BMP6,BMP8A,BMP8B,FZD7

Table 4.14. Pathways males HR vs males LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Oxidative Phosphorylation	0.002	0.07	-2.646	ATP5PB,ATP5PF,ATP5PO,COX17,NDUFA1,NDUFS5,UQCRB
Mitochondrial Dysfunction	0.007	0.05		ATP5PB,ATP5PF,ATP5PO,COX17,MT-ND6,NDUFA1,NDUFS5,UQCRB
Dermatan Sulfate Biosynthesis (Late Stages)	0.030	0.08		HS3ST1,SULT1A3/SULT1A4,UST
Chondroitin Sulfate Biosynthesis (Late Stages)	0.036	0.08		HS3ST1,SULT1A3/SULT1A4,UST
Role of Cytokines in Mediating Communication between Immune Cells	0.044	0.07		CXCL8,IL12A,IL15
Leukocyte Extravasation Signaling	0.048	0.04	0.816	ARHGAP5,CDH5,CLDN12,MMP24,PRKCI,PTK2,RAPGEF3

4.4.7 Biological Pathways differently modulated accordingly to biological sex: females

In this section I will describe the pathways associated with genes differently expressed in females for each comparison MDD, HR and LR.

4.4.7.1 Biological Pathways differently modulated in females MDD vs females HR adolescents

The pathways analysis was performed on the 399 genes differently expressed in the group of females MDD *vs* females HR (for the list of genes, see table 7E in Appendix E). From the analysis, 49 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.15 (see table 7G in Appendix G for the complete list of pathways). Thirteen pathways presented a positive z-score > 2, indicating that were up-regulated in MDD females compared with HR: 1) Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza (z-score 4.243); 2) Interferon Signaling (z-score 3); 3) Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses (z-score 2.646); 4) Systemic Lupus Erythematosus In B Cell Signaling Pathway (z-score 2.668); 5) TREM1 Signaling (z-score 2.646); 6) IL-17 Signaling (z-score 3); 7) Crosstalk between Dendritic Cells and Natural Killer Cells (z-score 2); 8) Acute Phase Response Signaling (z-score 2.236); 9) Hepatic Fibrosis Signaling Pathway (z-score 2.714); 10) Neuroinflammation Signaling Pathway (z-score 2.714); 11) HMGB1 Signaling (z-score 2); 12) Cardiac Hypertrophy Signaling (Enhanced) (z-score 2.138); 13) Wound Healing Signaling Pathway (z-score 2.333).

One pathway presented a negative z-score, indicating its down-regulation in MDD females: Coronavirus Pathogenesis Pathway (z-score -1.508).

Moreover, other pathways presented a positive z-score < 2, indicating that they were moderately up-regulated in MDD females compared with the HR group, specifically: Activation of IRF by Cytosolic Pattern Recognition Receptors; Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza; Role of PKR in Interferon Induction and Antiviral Response; Role of RIG1-like Receptors in Antiviral Innate Immunity; PCP (Planar Cell Polarity) Pathway; Salvage Pathways of Pyrimidine Ribonucleotides; Colorectal Cancer Metastasis Signaling; Role of WNT/GSK-3β Signaling in the Pathogenesis of Influenza.

4.4.7.2 Biological Pathways differently modulated in females MDD vs females LR adolescents

The pathways analysis was performed on the 471 genes differently expressed in the comparison females MDD *vs* females LR group (for the list of genes, see table 8E in Appendix E). From the analysis, 37 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.16 (see table 8G in Appendix G for the complete list of pathways). None of the 37 pathways were shown to be significantly up- or down-regulated in MDD females compared with LR females (meaning a positive z-score > 2 or a negative z-score < -2 respectively). However, there were some pathways up-regulated in MDD females, such as: Phagosome Formation; Breast Cancer Regulation by Stathmin1; G-Protein Coupled Receptor Signaling; RHOGDI Signaling; Natural Killer Cell Signaling; Sphingosine-1-phosphate Signaling; Osteoarthritis Pathway; Cardiac

Hypertrophy Signaling (Enhanced); Gαs Signaling; Adrenomedullin signaling pathway; Hepatic Fibrosis Signaling Pathway; Gαi Signaling and Cyclins and Cell Cycle Regulation.

On the other hand, a negative z-score was identified for the following pathways: CREB Signaling in Neurons; STAT3 Pathway; CDK5 Signaling; PAK Signaling; eNOS Signaling; Stearate Biosynthesis I; RAC Signaling and Pulmonary Fibrosis Idiopathic Signaling Pathway.

4.4.7.3 Biological Pathways differently modulated in females HR vs females LR adolescents

The pathways analysis was performed on the 504 genes differently expressed in the comparison females HR *vs* females LR (for the list of genes, see table 9E in Appendix E). From the analysis, 34 pathways were shown to be significantly modulated (p-value < 0.05), and those pathways enriched with more than three genes are reported in table 4.17 (see table 9G in Appendix G for the complete list of pathways).

One pathway presented a positive z-score > 2: 1) HGF Signaling (z-score 2); whereas two had a negative z-score < -2, indicating their down-regulation in HR females compared with the LR: 1) Inflammasome pathway (z-score -2.236); 2) Pyroptosis Signaling Pathway (z-score -2.449).

Table 4.15. Pathways females MDD vs females HR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.000	0.27	4.243	CCL2,CCL3,CXCL10,CXCL8,DDX58,EIF2AK2,IFIT2,IFIT3,IL1RN,IRF7,ISG15,MX1,OAS1,OAS2,OAS3,RSAD2,STAT2,TLR3
Interferon Signaling	< 0.000	0.26	3	IFI6,IFIT1,IFIT3,IFITM3,ISG15,JAK2,MX1,OAS1,STAT2
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.000	0.10	2.646	C1QB,CXCL8,DDX58,EIF2AK2,IFIH1,IRF7,OAS1,OAS2,OAS3,OSM,PIK3C2A,TLR3,TNFSF10,TNFSF13B
Systemic Lupus Erythematosus In B Cell Signaling Pathway	< 0.000	0.07	2.668	CD19,CD72,CD79A,CXCL8,FCGR2B,IFIH1,IFIT2,IFIT3,IRF7,ISG15,JAK2,OSM,PIK3C2A,STAT2,TLR3,TNFSF10,TNFSF13B
Agranulocyte Adhesion and Diapedesis	< 0.000	0.08		CCL2,CCL3,CCL8,CCR9,CLDN23,CXCL10,CXCL8,GNAI1,IL1RN,MMP24,MMP8,MYH11,XCL1
Granulocyte Adhesion and Diapedesis	< 0.000	0.07		CCL2,CCL3,CCL8,CCR9,CLDN23,CXCL10,CXCL8,GNAI1,IL1RN,MMP24,MMP8,XCL1
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.000	0.13	1.134	DDX58,DHX58,IFIH1,IFIT2,IRF7,ISG15,STAT2
TREM1 Signaling	0.001	0.10	2.646	CASP5,CCL2,CCL3,CXCL8,FCGR2B,JAK2,TLR3
Salvage Pathways of Pyrimidine Deoxyribonucleotides	0.001	0.33		APOBEC3A,APOBEC3B,TYMP
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza	0.001	0.10	1.89	CCL2,CXCL10,CXCL8,EIF2AK2,PLA2G4A,PLA2G4C,PLA2G7
Airway Pathology in Chronic Obstructive Pulmonary Disease	0.001	0.08		CCL2,CXCL8,LCN12,LCN2,MMP8,OSM,TNFSF10,TNFSF13B
Role of PKR in Interferon Induction and Antiviral Response	0.001	0.07	1.633	CASP5,DDX58,EIF2AK2,IFIH1,IL24,MARCO,MSR1,STAT2,TLR3
Coronavirus Pathogenesis Pathway	0.001	0.06	-1.508	BST2,CCL2,CXCL8,DDX58,IRF7,OAS1,OAS2,OAS3,SERPINE1,STAT2,TLR3
Atherosclerosis Signaling	0.003	0.07		CCL2,CXCL8,IL1RN,MSR1,PLA2G4A,PLA2G4C,PLA2G7,TPSAB1/TPSB2
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	0.004	0.05		APC2,CCL2,CXCL8,FZD5,IL17RC,IL1RN,JAK2,LRP6,OSM,PIK3C2A,TLR3,TNFSF13B,WNT10A,WNT5B
Communication between Innate and Adaptive Immune Cells	0.004	0.07		CCL3,CD79A,CXCL10,CXCL8,IL1RN,TLR3,TNFSF13B
IL-17 Signaling	0.005	0.06	3	CCL2,CXCL8,IL17RC,JAK2,LCN2,OSM,PIK3C2A,TNFSF10,TNFSF13B
TR/RXR Activation	0.006	0.07		ATP2A1,HP,PIK3C2A,PPARGC1A,STRBP,THRB
Role of RIG1-like Receptors in Antiviral Innate Immunity	0.007	0.11	1	DDX58,DHX58,IFIH1,IRF7

Crosstalk between Dendritic Cells and Natural Killer Cells	0.008	0.07	2	IL15RA,IL3RA,KIR3DL1,TLN2,TLR3,TNFSF10
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	0.009	0.06		APC2,FZD5,JAK2,PIK3C2A,TCL1A,WNT10A,WNT5B
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	0.011	0.13		CCL2,CCL3,LCN2
Role of Cytokines in Mediating Communication between Immune Cells	0.011	0.09		CXCL8,IL1RN,IL24,IL27
Role of IL-17F in Allergic Inflammatory Airway Diseases	0.014	0.09		CCL2,CXCL10,CXCL8,IL17RC
Neuroinflammation Signaling Pathway	0.015	0.04	2.714	CCL2,CCL3,CXCL10,CXCL8,GABRD,IRF7,JAK2,P2RX7,PIK3C2A,PLA2G4A,PLA2G4C,TLR3
IL-17A Signaling in Gastric Cells	0.016	0.12		CXCL10,CXCL8,IL17RC
Retinoate Biosynthesis I	0.017	0.11		ALDH1A1,ALDH8A1,RDH10
Role of MAPK Signaling in the Pathogenesis of Influenza	0.020	0.07		CCL2,CXCL10,PLA2G4A,PLA2G4C,PLA2G7
Acute Phase Response Signaling	0.023	0.05	2.236	C1QB,C1R,C4BPA,HP,IL1RN,JAK2,OSM,SERPINE1
Complement System	0.023	0.10		C1QB,C1R,C4BPA
MSP-RON Signaling Pathway	0.023	0.08		CCL2,IL3RA,JAK2,PIK3C2A
Glucocorticoid Receptor Signaling	0.026	0.03		CCL2,CCL3,CXCL8,ESR1,HP,IL15RA,IL17RC,IL1RN,IL31RA,IL3RA,JAK2,MMP8,PIK3C2A,PLA2G4A,PLA2G4C,POMC,SERPINE1
PCP (Planar Cell Polarity) Pathway	0.028	0.07	1	FZD5,PRICKLE1,WNT10A,WNT5B
Role of IL-17A in Arthritis	0.030	0.07		CCL2,CXCL8,IL17RC,PIK3C2A
Airway Inflammation in Asthma	0.030	0.09		CCL2,CXCL8,OSM
Hepatic Fibrosis Signaling Pathway	0.030	0.04	2.714	APC2,CCL2,CCL3,CXCL8,FZD5,GNAI1,IL1RN,JAK2,LRP6,PIK3C2A,SERPINE1,SUCNR1,WNT10A,WNT5B
Salvage Pathways of Pyrimidine Ribonucleotides	0.034	0.06	1.342	APOBEC3A,APOBEC3B,CMPK2,EIF2AK2,NME4
B Cell Development	0.035	0.09		CD19,CD79A,RAG1
Colorectal Cancer Metastasis Signaling	0.036	0.04	1.414	FZD5,GNAI1,JAK2,LRP6,MMP24,MMP8,PIK3C2A,TLR3,WNT10A,WNT5B
Human Embryonic Stem Cell Pluripotency	0.036	0.05		APC2,FZD5,GNAI1,PIK3C2A,S1PR3,WNT10A,WNT5B
HMGB1 Signaling	0.040	0.05	2	CCL2,CXCL8,OSM,PIK3C2A,SERPINE1,TNFSF10,TNFSF13B
Role of WNT/GSK-3β Signaling in the Pathogenesis of Influenza	0.043	0.06	1	APC2,FZD5,WNT10A,WNT5B
IL-17A Signaling in Fibroblasts	0.043	0.08		CCL2,IL17RC,LCN2
Wound Healing Signaling Pathway	0.044	0.04	2.333	COL15A1,CXCL8,IL1RN,JAK2,MMP8,OSM,TNFSF10,TNFSF13B,TPSAB1/TPSB2
Cardiac Hypertrophy Signaling (Enhanced)	0.046	0.03	2.138	ATP2A1,CXCL8,FZD5,GNAI1,IL15RA,IL17RC,IL31RA,IL3RA,JAK2,

				OSM,PDE7B,PIK3C2A,TNFSF10, TNFSF13B,WNT10A,WNT5B
Basal Cell Carcinoma Signaling	0.047	0.06		APC2,FZD5,WNT10A,WNT5B

Table 4.16. Pathways females MDD vs females LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
FAK Signaling	< 0.000	0.05	0	ADGRA3,ADGRF3,ADGRL3,ADRA1B,BCAR3,CCR9,COL18A1,CSF2RB,CXCR1,CXCR2,ERBB2,FFAR2,FZD5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,IL3RA,ITGA7,ITGA9,ITGAV,LPAR2,MRAS,OXTR,PAK6,PDGFRB,S1PR3,TGFB2
Phagosome Formation	< 0.000	0.05	0.365	ADGRA3,ADGRF3,ADGRL3,ADRA1B,APBB1IP,CCR9,CXCR1,FCGR2A,FFAR2,FZD5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,ITGA7,ITGA9,ITGAV,LIMK2,LPAR2,MARCKS,MRAS,MYD88,MYH10,OXTR,PAK6,S1PR3,TLR2,TTN
CREB Signaling in Neurons	< 0.000	0.05	-0.2	ADCY10,ADCY2,ADGRA3,ADGRF3,ADGRL3,ADRA1B,BMP6,CCR9,CXCR1,FFAR2,FGFR4,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,NTRK2,OXTR,PDGFRB,S1PR3,TGFB2
Human Embryonic Stem Cell Pluripotency	0.001	0.08		BMP6,FGFR4,FZD5,GNAQ,GNG5,INHBA,MRAS,NTRK2,PDGFRB,S1PR3,SMAD1,TGFB2
STAT3 Pathway	0.001	0.08	-0.707	BMP6,CSF2RB,CXCR1,CXCR2,FGFR4,IL1B,IL3RA,MRAS,NTRK2,PDGFRB,TGFB2
Breast Cancer Regulation by Stathmin1	0.001	0.05	1	ADGRA3,ADGRF3,ADGRL3,ADRA1B,BMP6,CCR9,CXCR1,FFAR2,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,OXTR,PPP2R2C,PPP2R5A,S1PR3,TGFB2,TUBB3
G-Protein Coupled Receptor Signaling	0.001	0.05	1.134	ADCY10,ADCY2,ADGRA3,ADGRF3,ADGRL3,ADRA1B,BORCS8-MEF2B,CCR9,CXCR1,CXCR2,FFAR2,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,OXTR,PAK6,PDE7B,S1PR3,TTN,WWTR1
RHOGDI Signaling	0.003	0.07	0.816	CDH4,ESR1,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,LIMK2,MRAS,MYH10,PAK6,RHOB
GABA Receptor Signaling	0.006	0.08		ADCY10,ADCY2,GABRA5,GABRD,GABRR2,GNAQ,GNG5,MRAS
Mitochondrial L-carnitine Shuttle Pathway	0.009	0.18		ACSL1,ACSL6,CPT1B
Gαs Signaling	0.010	0.07	1.134	ADCY10,ADCY2,ADD2,GNAQ,GNG5,HCAR2,HCAR3,MRAS

Cysteine Biosynthesis III (mammalia)	0.016	0.14		CBS/CBSL,EEF1AKMT3,SUV39H2
Cardiac Hypertrophy Signaling (Enhanced)	0.016	0.04	0.728	ADCY10,ADCY2,ADRA1B,BORCS8-MEF2B,CSF2RB,CXCR1,CXCR2,EDA,FGF9,FGFR4,FZD5,GNAQ,GNG5,IL1B,IL3RA,ITGA7,ITGA9,ITGAV,MRAS,PDE7B,TGFB2
Adrenomedullin signaling pathway	0.017	0.06	0.333	ADCY10,ADCY2,ADM,GNAQ,IL1B,IL1RN,MRAS,SHF,TFAP2E,TTN
Gαi Signaling	0.017	0.06	1.134	ADCY10,ADCY2,CXCR2,GNAQ,GNG5,HCAR2,MRAS,S1PR3
CDK5 Signaling	0.018	0.07	-1.134	ADCY10,ADCY2,LAMC1,MRAS,NTRK2,PPP2R2C,PPP2R5A
PAK Signaling	0.019	0.07	-1	ITGA7,ITGA9,ITGAV,LIMK2,MRAS,PAK6,PDGFRB
Hepatic Cholestasis	0.019	0.06		ABCB1,ADCY10,ADCY2,EDA,ESR1,FGFR4,IL1B,IL1RN,MYD88,TGFB2
Natural Killer Cell Signaling	0.021	0.05	1	COL18A1,FCGR2A,HSPA1A/HSPA1B,HSPA6,KIR3DL2,KLRC2,LIMK2,MRAS,MYD88,PAK6
Sphingosine-1-phosphate Signaling	0.022	0.06	1.134	ADCY10,ADCY2,CASQ1,GNAQ,PDGFRB,RHOB,S1PR3
PI3K/AKT Signaling	0.025	0.05		CSF2RB,CXCR1,CXCR2,IL3RA,ITGA7,ITGA9,ITGAV,MRAS,PPP2R2C,PPP2R5A
Osteoarthritis Pathway	0.026	0.05	1.134	CASQ1,CXCR2,FZD5,IL1B,ITGA7,ITGA9,ITGAV,PTCH1,S1PR3,SMAD1,TLR2
eNOS Signaling	0.030	0.06	-0.378	ADCY10,ADCY2,CCNA1,ESR1,GNAQ,HSPA1A/HSPA1B,HSPA6,LPAR2
IL-1 Signaling	0.030	0.07		ADCY10,ADCY2,GNAQ,GNG5,MRAS,MYD88
Stearate Biosynthesis I (Animals)	0.032	0.09	-1	ACSL1,ACSL6,BDH2,DHCR24
Agranulocyte Adhesion and Diapedesis	0.032	0.05		CCL22,CCR9,CLDN9,CXCL16,CXCR1,CXCR2,IL1B,IL1RN,MYH10
Axonal Guidance Signaling	0.034	0.04		ADAMTS6,BMP6,ERBB2,FZD5,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,LIMK2,MRAS,NTRK2,PAK6,PTCH1,SEMA4G,SHANK2,TUBB3,UNC5B
PPARα/RXRα Activation	0.036	0.05	0.707	ACOX1,ADCY10,ADCY2,CPT1B,GK,GNAQ,IL1B,MRAS,TGFB2
Dopamine Receptor Signaling	0.036	0.07		ADCY10,ADCY2,NCS1,PPP2R2C,PPP2R5A
Phospholipase C Signaling	0.041	0.05	1.89	ADCY10,ADCY2,BORCS8-MEF2B,FCGR2A,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,MARCKS,MRAS,RHOB
Cardiac β-adrenergic Signaling	0.041	0.05	-0.816	ADCY10,ADCY2,GNAQ,GNG5,MRAS,PDE7B,PPP2R2C,PPP2R5A
Fatty Acid Activation	0.043	0.15		ACSL1,ACSL6

Hepatic Fibrosis Signaling Pathway	0.046	0.04	0.905	COL18A1,FTH1,FZD5,IL1B,IL1RN,ITGA7,ITGA9,ITGAV,LRP5,MRAS,MYD88,PDGFRB,PTCH1,RHOB,TGFB2,TTN
Sertoli Cell-Sertoli Cell Junction Signaling	0.047	0.05		ADCY10,CLDN9,ITGA7,ITGA9,ITGAV,MRAS,PALS2,TJP1,TUBB3
Superpathway of Methionine Degradation	0.049	0.09		CBS/CBSL,EEF1AKMT3,SUV39H2
RAC Signaling	0.050	0.05	-1	ITGA7,ITGA9,ITGAV,LIMK2,MCF2L,MRAS,PAK6

Table 4.17. Pathways females HR vs females LR (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
STAT3 Pathway	< 0.000	0.10	1.134	BMPR1A,CDKN1A,CISH,FGFR2,IGF1,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,MAP3K20,SOCS3
Inflammasome pathway	< 0.000	0.25	-2.236	AIM2,CASP1,CASP5,NLRC4,NLRP3
Osteoarthritis Pathway	< 0.000	0.07	-0.577	ALPL,ANKH,BMPR1A,CASP1,CASP4,CASP5,CEBPB,DDIT4,FZD7,IL18RAP,IL1RL1,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
PI3K/AKT Signaling	0.001	0.07	1	BCL2L1,CCND1,CDKN1A,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Caveolar-mediated Endocytosis Signaling	0.003	0.10		CAV1,CD55,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Granulocyte Adhesion and Diapedesis	0.005	0.07		CCL23,CCL3L1,CCR9,CXCL16,CXCL6,HRH4,IL18RAP,IL1RL1,IL36A,SDC1,SDC2
PTEN Signaling	0.006	0.07	-1.633	BCL2L1,BMPR1A,CCND1,CDKN1A,FGFR2,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Eicosanoid Signaling	0.007	0.10		AKR1C3,ALOX15,ALOX15B,CYSLTR2,PLAAT5,PRDX6
Cardiac Hypertrophy Signaling (Enhanced)	0.008	0.05	1.213	ACE,ADCY10,ATP2A1,CACNB4,FGFR2,FZD7,IGF1,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL36A,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MAP3K20,OSM,PDK1,PIK3R6
Complement System	0.009	0.13	1	C3,CD55,CFD,CR1
TREM1 Signaling	0.011	0.09	-1.633	CASP1,CASP5,IL1RL1,NLRC4,NLRP3,TREM1
IL-10 Signaling	0.012	0.09		BLVRA,IL18RAP,IL1RL1,IL36A,IL4R,SOCS3
IL-13 Signaling Pathway	0.014	0.07	0.378	ALOX15,ALOX15B,BCL2L1,CXCL6,IL4R,PIK3R6,SOCS3
ID1 Signaling Pathway	0.016	0.06	0.905	BCL2L1,BHLHA15,BMPR1A,CAV1,CCND1,CDKN1A,FGFR2,GSPT1,PIK3R6,RAP1GAP,TGM2
Iron homeostasis signaling pathway	0.019	0.07		ABCB10,ATP6V0C,BMPR1A,CDC34,FECH,FTH1,HFE,SLC25A37
Phagosome Formation	0.022	0.04	0.816	ADORA3,C3,CCR9,CLEC4D,CR1,CYSLTR2,FCER1G,FCGR3A/FCGR3B,FZD7,GPR146,HRH4,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MARCO,MYO10,P2RY2,PIK3R6,PLAAT5,PRDX6,PTGDR2,VTN
JAK/STAT Signaling	0.023	0.07	0.816	BCL2L1,CDKN1A,CEBPB,CISH,PIK3R6,SOCS3
HGF Signaling	0.025	0.06	2	CCND1,CDKN1A,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,PIK3R6

Pyroptosis Signaling Pathway	0.026	0.07	-2.449	AIM2,CASP1,CASP4,CASP5,NLRC4,NLRP3
Regulation of Cellular Mechanics by Calpain Protease	0.027	0.07		CCND1,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Sertoli Cell-Sertoli Cell Junction Signaling	0.028	0.05		ADCY10,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MAP3K20,SPTA1,TUBB6,YBX3
Th2 Pathway	0.028	0.06	0	HLA-DRB5,IL12RB2,IL1RL1,IL4R,NOTCH4,PIK3R6,PTGDR2,SOCS3
Type II Diabetes Mellitus Signaling	0.032	0.06		ACSF2,ACSM1,ADIPOR1,CACNB4,CEBPB,PIK3R6,SMPD3,SOCS3
Retinoate Biosynthesis I	0.036	0.11		AKR1C3,ALDH1A2,HSD17B6
FAK Signaling	0.037	0.04	1.877	ADORA3,CCND1,CCR9,CYSLTR2,FCER1G,FZD7,GPR146,HRH4,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,P2RY2,PIK3R6,PTGDR2,SOCS3
Th1 and Th2 Activation Pathway	0.038	0.05		HLA-DRB5,IL12RB2,IL1RL1,IL27,IL4R,NOTCH4,PIK3R6,PTGDR2,SOCS3
Endocannabinoid Cancer Inhibition Pathway	0.044	0.06	-1.414	ADCY10,CASP1,CASP4,CASP5,CCND1,CDKN1A,PIK3R6,SMPD3
LPS/IL-1 Mediated Inhibition of RXR Function	0.048	0.05		ABCB9,ACSF2,ACSM1,ALDH1A2,CHST10,CRAT,GSTM2,IL18RAP,IL1RL1,IL36A

4.5 Comparison between microarrays and RNA-Seq

Given the fact that two different omics techniques were used, a correlation analysis was performed to investigate the correlation level of those two techniques. Specifically, quantitative comparison of the relative raw expression profile of the common genes present in each platform was computed by using Spearman's correlation. The correlation was performed with regards to the following comparison: MDD *vs* HR, MDD *vs* LR, and HR *vs* LR considering the entire cohort.

For the MDD *vs* HR comparison, 14821 genes were identified as differently modulated and in common between both RNA-Seq and microarray platform, and the Spearman's correlation computed between the fold-changes was 0.173 ($p < 0.01$). When considering only the DEGs with an unadjusted p-value lower than 0.05 and in common between both techniques, 52 genes were observed to be in common and they showed a correlation coefficient of 0.709 ($p < 0.01$).

For the MDD *vs* LR comparison, 14898 genes were identified as differently modulated in both RNA-Seq and microarray platform, and the Spearman's correlation computed between the fold-changes was 0.266 ($p < 0.01$). When considering only the DEGs with an unadjusted p-value lower than 0.05 and in common between both techniques, 104 genes were observed to be in common and they presented a correlation coefficient of 0.761 ($p < 0.01$).

For the HR *vs* LR, 14907 genes were identified as differently modulated in both RNA-Seq and microarray platform, and the Spearman's correlation computed between the fold-changes was 0.122 ($p < 0.01$). When considering only the DEGs with an unadjusted p-value lower than 0.05 and in common between both techniques, 14

genes were observed to be in common and they presented a correlation coefficient of 0.284 ($p > 0.05$).

5. Discussion

In this section, I will discuss the main findings reported in this doctoral thesis. I will start by briefly discussing the results of the quality control analysis, as important starting point for feasibility and quality of analyses for future studies recruiting in LMICs. It will follow the discussion of the biological results of the genome-wide gene expression results performed with both Affymetrix microarrays and RNA Sequencing techniques. I will firstly discuss the differences and similarities between the two techniques, by discussing pros and cons, and by also considering the results of the correlation analysis performed between them. It will follow the discussion of the biological results, starting from analysing the DEGs and then by focusing the attention mainly on the results of the pathways analysis; both results will be discussed by focusing first on the entire cohort and then on the biological-sex driven results. Lastly, I will discuss strengths and limitations of my doctoral thesis in the context of the IDEA project, and I will provide possible future directions to implement the knowledge for study of adolescent depression specifically in LMICs.

5.1 Summary of findings

In this doctoral thesis, I performed a genome-wide gene expression analysis from blood samples of a cohort of 150 Brazilian adolescents stratified based on an increased risk of depression (Kieling et al., 2021). The cohort was constituted by 50 adolescents with a current diagnosis of depression and classified at high risk of developing the disorder accordingly to the composite risk score; 50 adolescents without a diagnosis of depression and at high risk of developing it, and 50 classified as low risk. Peripheral blood samples were collected from each adolescent to isolate nucleic acid for performing genome-wide gene expression analysis. The gene expression analysis was followed by the analysis of the genes differently expressed (DEGs) among the three risk groups as well as the pathways analysis to identify biological mechanisms underpinning the onset and the risk of depression.

The results from the genome-wide gene expression analysis with both microarrays and RNA-seq showed a possible modulation toward an activation of pathways associated with inflammation and immune system in adolescents with depression compared with their non-depressed peers. This result suggested that an activated status of inflammation and immunity might map the presence of depression in adolescents. On the other hand, a panel of pathways with a common biological pattern was not observed when comparing the non-depressed adolescents at high or low risk of developing MDD, and fewer biological differences were observed between these two groups, suggesting that no such stronger biological differences were detected within HR and LR groups.

The same analysis was also performed considering males and females separately, to investigate biological-sex driven differences in the pathways mapping adolescent depression. Overall, both males and female adolescents with depression showed an up-regulation of pathways associated with inflammation compared with both the non-depressed groups, similarly to what was observed in the results related to the entire cohort. However, inconsistent results were observed in boys with depression according to the two different techniques used. On the other hand, similarly to what observed for the entire cohort, no biological significant differences in males and females from HR and LR groups were detected.

A summary of the results corresponding to each aim in the paragraph "Aims and Hypothesis" can be found below.

The first aim was the identification of biological pathways mapping the presence and the risk of developing adolescent depression in Brazilian adolescents by using the microarray technique via Affymetrix Gene Atlas Platform. Being a hypothesis free approach, no *a priori* specific genes or pathways were predicted to be differently modulated among MDD, HR or LR adolescents. However, I predicted the existence of differences in terms of biological pathways differently modulated among the three groups. Indeed, I observed an up-regulation of inflammatory and immune system's pathways in adolescents with depression when compared with both risk groups, suggesting a possible role of inflammation as a characterizing factor of adolescent depression. On the other hand, I did not observe significant differences among the HR and LR adolescents, suggesting that biological differences were more evident in association with the presence rather than the increased risk of adolescent depression.

The second aim was the identification of differences underlying the presence of MDD and the risk of developing adolescent depression in Brazilian adolescents potentially driven by biological sex by using the microarray Affymetrix technique. Given the higher incidence of depression in females compared with males, I hypothesized that different pathways might be differently regulated in females and males with depression or accordingly to the risk. Indeed, similarly to the results observed for the entire cohort, a modulation toward an activation of inflammation resulted also when considering males and females separately, and the effect appeared to be more robust in males with depression rather than females. On the other hand, no significant biological differences were observed when comparing the risk groups, as previously described for the analysis on the entire cohort.

The third and the fourth aims were similar to the first two ones, but this time the hypothesis was focused on the results from the second genome-wide gene expression approach used, which was RNA Sequencing. Thus, the aims were the identification of biological pathways mapping the presence of MDD and the risk of adolescent depression in Brazilian adolescents, as well as identifying possible biological sex differences by using the RNA-Seq technique. Similarly to aims one and two, being a hypothesis free approach, no *a priori* specific genes or pathways were predicted to be differently modulated among the MDD, HR or LR adolescents or driven by biological sex. However, I predicted the existence of differences in terms of biological pathways differently regulated among the three groups as well as differences driven by biological sex.

Overall, an up-regulation of pathways associated with inflammation and immune system resulted in depressed adolescents compared with the HR group, whereas a less strong modulation was observed when compared the MDD group with the LR group. These results suggest a possible role of inflammation as a biological signature mapping the presence of depression in adolescents but not associated with the increased risk of developing the disorder, as no inflammation related pathways were observed when comparing HR and LR adolescents. Moreover, the stronger activation in MDD when compared with HR rather than when compared with LR, might suggest e role of inflammation in the onset of depression from a high-risk condition, as the MDD adolescents also met criteria for high risk of developing depression accordingly to the risk score.

When investigating the role of biological sex, inflammation-related pathways were up-regulated in females with depression compared with HR females, whereas no such differences were observed in the male counterparts, suggesting this time a major effect of inflammation in females. Moreover, independently from biological sex, no major biological differences were observed in the comparison between the two risk groups.

5.2 Feasibility of conducting rigorous biological research for adolescent depression in under-represented and low-resource settings

A global challenge in mental health research is the development of biomarkers and endophenotypes that can accurately identify the risk for depression. Although technologies to develop comprehensive pictures of depression risk are widely available in HICs, there is limited opportunity to develop similarly comprehensive pictures in poorer settings, also due to limited equipment and qualified personnel. As part of the IDEA Project, assessing and understanding the feasibility of collecting biological samples in adolescents from Brazil was considered an important objective to open the opportunities of conducting biologically enriched studies in settings with cultural, social, and economical setting poorest than Brazil in the future (the so-called LMICs). Testing the feasibility of collection and handling of biological samples from adolescents in Brazil was considered an essential starting point for future studies in LMICs such as Nepal and Nigeria that were part of the project.

Moreover, assessing the quality of the samples collected was of paramount importance to ensure a good quality of the subsequent genome-wide gene expression analysis, which require a very good quality of RNA.

For the purpose of the study, the process of working alongside the Brazilian team to better adapt UK Standard Operating Procedure (SOP) to the local setting was proven an essential step to ensure feasibility of collection and handling of biological samples for the gene expression analyses. The quality checks analysis of the RNA samples showed that most samples had a RIN value higher than 8, indicating very good quality. The quality checks performed on the raw data from Affymetrix and NextSeq550

showed good quality runs, confirming that good quality of the starting RNA was a requirement for the subsequent analysis. My analyses and the work conducted as part of my doctorate clearly show feasibility of rigorous biological research for adolescent depression in under-represented and low-resource settings and support the importance of co-development of SOP with the local settings to ensure good quality of the biological samples for subsequent laboratory analyses. This approach and our findings support feasibility and importance of continuing and/or building capacity for future biological psychiatry research in LMICs such as Nepal and Nigeria.

5.3 Transcriptional differences in MDD, HR and LR adolescents

The first results provided by the Affymetrix data are the lists of genes differently modulated in each of the three comparisons: MDD *vs* HR, MDD *vs* LR, and HR *vs* LR. As previously described in the Methods section, each gene list has been filtered for Fold-Change and p-values cut-offs, specifically FC ±|1.2|, $p<0.05$, and q-value < 0.05 for both microarray and RNA-Seq techniques. In our research group, similar FC and unadjusted p-value cut-offs values were widely used for the microarrays analysis as indicative of biological significance for the identification of both genes significantly differently modulated as well as for the subsequent pathways analysis (Anacker et al., 2013; Borsini et al., 2018; Cattaneo et al., 2019; Cattaneo et al., 2018; Hepgul et al., 2016; X. Li et al., 2017). On the other hand, this was the first time that such cut-offs for the RNA-Seq were applied since this doctoral thesis represents the first time we did perform -omics analysis by using RNA-Seq. The data that will be discussed below were all generated by using the cut-offs values described, as already reported in the Results section.

In the following paragraphs I will discuss the genes differentially expressed (named DEGs) specifically by considering those surviving the cut-offs of p-value < 0.05 and FC ±|1.2|. Specifically, I will discuss the overall number of DEGs for both techniques by focusing on differences and similarities between the different comparison. On the other hand, the DEGs also surviving the FDR correction (q-value < 0.05) will be discussed separately, as they represent a very small number and were identified only for RNA-Seq analysis and not for microarrays, as previously reported in the Results section.

For the microarrays analysis, in the comparison between adolescents with depression and HR participants I found 79 genes differently expressed, and 89.9% were up-regulated in the MDD group compared with HR adolescents. In the comparison MDD *vs* LR, I found 23 genes differently expressed and again the majority (60.8%) were up-regulated in the MDD group compared with LR. Lastly, only 11 genes were differently modulated in HR adolescents compared with the LR group, and 9 out of 11 genes were down-regulated in HR adolescents. By looking at these results, we can observe that the comparison MDD *vs* HR accounted for the highest number of DEGs, whereas the comparison HR *vs* LR accounted for the lowest. This result can be indicative of a higher variability in terms of gene expression in the depressed group compared with the risk groups, suggesting that a stronger biological difference might be attributable to the actual presence of depression rather than to the only risk of developing it.

On the other hand, when analyzing the microarray results separately for males and females, the differences in terms of DEGs was more robust, especially in the comparison of male adolescents with depression *vs* HR males which accounted for 592 genes differently expressed, whereas in the same comparison for the female counterparts the DEGs were only 42. This result might suggest a higher variability in terms of gene expression in males with depression compared with females with depression; however, to my knowledge no previous studies reported such similar differences in terms of DEGs in -omics approaches.

I observed differences within the comparisons also in terms of the number of genes up- or down-regulated. In the entire cohort, the comparison MDD *vs* HR and MDD *vs* LR showed a higher number of up-regulated genes (accounting for 89.9% and 60.8%

of the total, respectively), and similar results were observed also in the analysis in only males (90.4% and 80 % respectively) and females (71.4% and 68.6% respectively). On the other hand, the vast majority of DEGs in the comparison HR *vs* LR were down-regulated, and this was observed in the entire cohorts (81.8%), in males (74%) and in females (81.4%). These results might indicate that the presence of depression is accompanied by changes in gene expression in adolescents, both males and females, as suggested by the very high number of up-regulated genes.

Regarding the number of DEGs from RNA-Seq, fewer differences within the comparisons were observed in terms of numerosity as well as general up- or down-regulation. In the entire cohort, 313 genes were differently expressed in MDD *vs* HR comparison and 55.3% were up-regulated in adolescents with depression. I then observed a higher number of DEGs in the comparison MDD *vs* LR, as 461 genes passed the cut-off values, and among them 44.7% were up-regulated in the depressed group, showing a different result compared to what I observed in the same comparison in the microarray results. Lastly, the comparison HR *vs* LR accounted for a lower number of DEGs, 192 of which 67.2% were down-regulated in the HR group.

When analyzing the RNA-Seq results divided by biological sex, I observed 310 DEGs in males with depression compared with HR, and 377 compared with LR. In the former comparison, the number of up- and down-regulated genes was almost identical, whereas in the latter the majority were up-regulated (62.9%). For the female counterpart, among the 399 DEGs in the MDD *vs* HR comparison, the majority was up-regulated (65.9%), whereas opposite results were observed in MDD *vs* LR which accounted for 471 DEGs and 55.6% were down-regulated. An opposite trend,

compared with microarray results resulted in the HR *vs* LR comparison in both males and females, as for both sexes I did not found a reduction in terms of numbers of DEGs compared with the other two comparisons. Regarding the direction of the gene expression, in males most genes were up-regulated in HR adolescents (around 60%) opposite to what was observed in the microarray results, whereas in HR females 60,9% of DEGs were down-regulated, accordingly to what previously observed for the microarray results.

5.4 Differences in DEGs identified by microarray and RNA-SEQ

Overall, I observed differences in the number of DEGs within the same comparisons in the two different -omics techniques. This difference is in line with the current knowledge about the comparability of Affymetrix and RNA-Seq, as similar results were already observed in literature.

A quantitative comparison of the relative raw expression profile of the common genes resulted from both platforms was computed by using SPSS software, and the results were reported in the results paragraph 4.5. Overall, the correlation coefficients were low when considering the entire panel of genes in common between the two techniques without applying any cut-offs value (ranging from 0.122 to 0.266). On the other hand, when considering only the genes who survived the cut-off value of unadjusted p-value lower than 0.05, the Spearman's coefficient was higher (0.709 for the MDD *vs* HR, and 0.761 for MDD *vs* LR), suggesting that the genes most significantly modulated and in common between the two platforms have a much higher correlation if compared with the total genes in common. Similar correlation coefficients were observed also in the study of Rao and colleagues, and further studies suggested such correlation index (Rao et al., 2018). These differences in the correlation results might be due to the intrinsic differences of the two techniques, and specifically to the limitations of the microarrays approach. However, the higher correlation that is observed only when comparing the genes more significantly differently modulated, might suggest that those genes could reasonably represent the strongest results, as not only in common between the two techniques but also highly correlated.

Moreover, in line with the results reported in this doctoral thesis, several studies reported higher numbers of genes detected as differently expressed in RNA-Seq compared with microarray (Munster SK, 2018; Perkins et al., 2014; Rao et al., 2018; S. Zhao, Fung-Leung, Bittner, Ngo, & Liu, 2014), especially for those that were down-regulated (S. Zhao et al., 2014).

As previously suggested, the reasons underpinning these differences lies in the intrinsic differences between the two techniques, that indeed highlighted the limitations of microarrays, which are mostly overcome by RNA-Seq. In line with the results presented in this doctoral thesis, previous studies showed that RNA-Seq is more sensitive in detecting genes with very low expression and more accurate in detecting expression of extremely abundant genes (S. Zhao et al., 2014). In contrast, microarrays measure the expression of thousands of genes in a sample by quantifying the hybridization of fragmented cDNA to a set of complementary probes specifically designed to detect a set of genes or transcripts. The two most significant drawbacks associated with microarrays are a non-specific binding and a signal saturation, which can negatively affect the detection of genes both highly and lowly expressed. The non-specific binding leads to background signals which can prevent the detection of lowly expressed genes, while highly expressed transcripts may saturate the fluorescent signals, thus compromising their detection (Binder & Preibisch, 2005; Marioni, Mason, Mane, Stephens, & Gilad, 2008). On the other hand, RNA-seq does not present such disadvantages, and it is able to detect more DEGs than microarrays, explaining thus also the results presented in this doctoral thesis as well as those already published in the literature (S. Zhao et al., 2014). Furthermore, as the microarray technique is based on the binding of complementary cDNA fragments to specific probes on the

microarray itself, this approach is based on prior knowledge of the transcriptome and therefore microarrays can only interrogate a subset of known or predicted transcripts. Conversely, RNA-Seq does not need *a priori* knowledge of the transcriptome as it investigates the entire transcriptome, thus allowing the identification of more targets. The low correlation between Affymetrix and RNA-Seq observed in this doctoral thesis, might be explained also considering the observation of Perkins and colleagues. Specifically, they hypothesized that most of the genes differently expressed only in the microarray and not in the RNA-Seq might be false positive, as they had low FC values and thus the apparent significant change in expression might be due to non-specific binding (Perkins et al., 2014; Z. Wang, Gerstein, & Snyder, 2009). Consequently, this drawback of the microarrays, together with the high background noise, might explain the low Pearson's correlations resulted when comparing the fold-changes of all the genes in common, as many of them presented a fold-change close to one, and thus they might be false positive. On the other hand, when considering only the genes most significantly modulated, the correlation coefficient is much higher, in line with previous literature studies.

As the RNA-Seq is more sensitive in detecting genes with very low expression and more accurate in detecting also extremely abundant genes, greater fold-change ranges can be detected in RNA-seq than in microarrays (Munster SK, 2018; S. Zhao et al., 2014), and this was observed also in the results of this doctoral thesis.

Overall, the results presented in this thesis showed a good correlation between the fold-changes of microarrays and RNA-Seq for the same genes when applying a p-value cut-off considering only the genes in common and most significantly differentially

expressed. However, both the data from this thesis (such as the correlation coefficient) as well as the literature demonstrated that RNA-Seq technique is more accurate, and it can provide a wider range of DEGs given the lack of disadvantages that were widely observed and described for the microarrays. Considering these results, the future analysis for the IDEA follow-up as well as the future -omics analysis that will be performed in our research group, will be conducted by using RNA-seq rather than microarrays, as it was widely observed to have less limitation and to be more accurate. Moreover, also the following discussion will be mainly focused on the results from the RNA-Seq, although also the microarrays results will be discussed given the good correlation observed for the most significant DEGs.

5.5 Transcriptional differences and focus on the top genes differently modulated in MDD, HR and LR adolescents

As already described in the results section, among the genes identified to be differently expressed based on the cut-off values of p-value < 0.05 and FC ± |1.2|, none of those from the microarray analysis survived the FDR correction presenting a q-value < 0.05. On the other hand, some of DEGs genes identified from the RNA-Seq and based on the same cut-offs values previously described, survived the FDR correction and present a q-value < 0.05. Moreover, other DEGs from the RNA-seq results were shown to present a q-value < 0.1, which is a less strict cut-offs but already used in literature. In this paragraph, a brief discussion will follow to pinpoint possible roles of those genes presenting a q-value < 0.05 or a q-value < 0.1.

Before discussing the role of these genes, it is noteworthy to mention that such paucity of genes identified as differently expressed and that survived the FDR correction, is in line with other studies in literature, detecting few or no genes differently expressed in similar conditions. For instance, similar results were observed in the very recent study of Cole and colleagues published in 2021 (J. J. Cole et al., 2021). The authors performed RNA-Seq analysis on PBMC of 44 adult control individuals, 94 adult treatment-resistant depressed patients, 47 adult depressed treatment-responsive and 46 adult depressed untreated individuals; then, they performed differential expression analysis by using DESeq2 to characterize any differences between the healthy controls and each of the MDD groups. Their differential analysis showed only one gene to differ significantly (q-value < 0.01)

between HC and total MDD and none between HC and MDD sub-groups. Although their cut-off was tighter than the one used in this doctoral thesis, after investigating the distribution of the p-values among the different comparisons and by randomizing cases and controls and performing over representation analysis, they concluded that there was no justification for relaxing the p-value threshold in their data, and that there were no differences between control individuals and MDD groups. These results are comparable to previous transcriptomic studies in whole blood which also found no signature at adjusted $p < 0.05$ using larger sample numbers (Mostafavi et al., 2014). Moreover, other studies used different cut-offs values, ranging from $p < 0.1$ to $p < 0.25$ (J. J. Cole et al., 2021).

Although only a few genes presented a q-value <0.05, for this discussion further investigations were performed to hypothesize their possible role in adolescent depression by considering the current literature. As previously mentioned, in this paragraph also the genes with a q-value < 0.1 will be described, as a significant difference in terms of q-value was observed between those genes and those with q-value >0.1.

Entire Cohort, MDD *vs* LR. In the analysis of the entire cohort of adolescents with depression versus LR adolescents, the gene NRCAM was down-regulated in the depressed group (FC -1.62, q-value 0.089). This gene encodes for the Neuronal Cell Adhesion Molecule, which is part of the Cell Adhesion Molecules (CAMs) family, transmembrane proteins located on the cell surface and involved in the binding with other cells (Walmod, Kolkova, Berezin, & Bock, 2004). CAMs are involved in several

vital processes controlling cell proliferation, activation, migration, and survival (McKeown, Wallace, & Anderson, 2013). Moreover, recent studies showed that CAMs play a role in several neurological and psychiatric diseases, such as Alzheimer's disease, schizophrenia, and depression (Brennaman & Maness, 2010; Sandi & Bisaz, 2007). CAMs were also shown to be involved in the inflammatory and immune response, suggesting a further role in depression (Lee et al., 2016). Specifically, NRCAM protein levels were investigated in depressed patients, showing heterogenous results. In line with the findings of this doctoral thesis, showing a down-regulation of NRCAM in adolescents with depression compared with LR non-depressed individuals, a down-regulation was shown in the CSF of depressed patients compared with healthy controls (Hidese et al., 2017). Moreover, pre-clinical studies showed that NRCAM-deficient mice exhibited depressive-like behavior (Aonurm-Helm et al., 2008), and chronically stressed rats showed a reduced NRCAM protein levels in the hippocampus (Venero et al., 2002). On the other hand, NRCAM was also shown to be up-regulated in plasma of depressed patients compared with controls (W. Liu et al., 2021) as well as in CSF of patients with unipolar depressive disorder (Poltorak et al., 1996). Consequently, further investigation might be needed specifically in blood samples of depressed patients, as most of the studies focused on CSF samples.

A further interesting gene is the Complement Component 4 Binding Protein Alpha (C4BPA), which was down-regulated in adolescents with depression compared with the LR group (FC -3.65, q-value 0.053). Complement is part of the human immune system that protects the body against the invasion and proliferation of various pathogens (Tichaczek-Goska, 2012). Accordingly, some researchers suggested that deficiencies in the complement classical pathway may be involved in autoimmune

related mechanisms that contribute to the onset of mental disorders. Specifically, within the complement system, some evidence indicated that dysfunction of the complement protein C4b may be involved in the alterations of the innate and adaptive immune systems in schizophrenia (Mayilyan, Dodds, Boyajyan, Soghoyan, & Sim, 2008). In line with the down-regulation observed in this thesis, the study performed by Focking and colleagues showed reduced levels of C4BPA protein in the plasma of individual at age 12 who reported psychotic experience at age 18 compared with control adolescents; on the other hand, in the same study other complement proteins were shown to be up-regulated in the same individuals (Focking et al., 2021). However, results regarding the role on C4BPA in schizophrenia were also inconsistent (S. Wang et al., 2015; Yue et al., 2011). On the other hand, few studies were conducted investigating the role of complement in depression. Indeed, reduced levels of C4BPA were associated with post-partum depression in the study of Mehta and colleagues (Mehta et al., 2021). Moreover, the complement factor H (CFH) was shown to confer susceptibility to depression in the Han Chinese population, as reported in the study of Zhang and colleagues showing reduced mRNA and protein levels of CFH in depressed individuals compared with controls (C. Zhang et al., 2016). On the other hand, Wei and colleagues showed increased levels of complement component 4 in peripheral blood of a cohort of adult Han Chinese patients with depression compared with controls as well as reduced levels after antidepressant treatment (Wei et al., 2018). Similar results were observed also by Kronfol and Housed, as increased levels of complement component 3 and 4 were measured in blood of patient with depression compared with control individuals (Kronfol & House, 1989) Hence, further investigation of the role of the complement system and C4PBA might shed light on its role in depression.

With regards to the other genes reporting a q-value < 0.1 PLS3, STRBP, and TBC1DH3, to the current knowledge no studies investigated their role in mental health disorders. The gene PLS3 encodes for the plastin 3 protein, also known as T-plastin or fimbrin. Studies showed that PLS3 knockout or mutation caused osteoporosis, whereas its overexpression induced osteoarthritis and several types of cancer. Moreover, the overexpression of PLS3 was shown to be protective in neuromuscular disorder, such as spinal muscular atrophy and amyotrophic lateral sclerosis (Wolff et al., 2021).

The gene STRBP encodes for the Spermatid Perinuclear RNA Binding Protein, a microtubule-associated RNA-binding protein. STRBP was shown to be mainly involved in spermatogenesis and sperm function, and to play a role in regulation of cell growth (Pires-daSilva et al., 2001).

Lastly, the gene TBC1DH3 encodes for the TBC1 Domain Family Member 3H protein, which acts as a GTPase activating protein for RAB5, a key factor in regulating early endocytosis (Hodzic et al., 2006).

Males, MDD *vs* LR. In the analysis of male adolescents, in the comparison MDD *vs* LR, the gene Cytochrome P450 Family 26 Subfamily B Member 1 (CYP26B1) was down-regulated in the depressed group (FC -21.32, q-value 0.070). This gene encodes a member of the cytochrome P450 superfamily, that are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. CPY26B1 is a critical regulator of all-trans retinoic acid levels by their specific inactivation, and this function was studied in pre-clinical studies in relation to depressive-like behavior. Knockdown of CP26B1 in the rats' nucleus

accumbens shell increased depression-related behavior, whereas decreased anxiety-like behavior (Y. Zhang et al., 2019). Similarly, increased retinoic acid (RA) signaling reached by knocking down the RA degradation enzyme CYP26B1 within the nucleus accumbens shell increased cocaine-taking and -seeking behaviors in rats exposed to environmental enrichment (C. Zhang et al., 2016). Moreover, in this study environmentally enriched rats also showed a protective depression phenotype, therefore high RA signaling and thus reduced CYP26B1 levels (in line to the results of this doctoral thesis as well) may play a similar role in inducing depression-related behavior in this pre-clinical model. Another pre-clinical study showed that knockout of CYP26B1 in mice during postnatal life were associated with severe health problem and increased inflammation, via inducing an increase of all-trans retinoic acid which in turn triggered inflammation (Snyder et al., 2020).

The gene Defensin Alpha 1 (DEFA1) was up-regulated in male adolescents with depression compared with the LR counterpart (FC 15.06, q-value 0.080). The protein defensin alpha 1 is found in the microbicidal granules of neutrophils and plays a role in phagocyte-mediated host defense. To my knowledge, no studies investigated DEFA1 in association with depression; however, its up-regulation in adolescents with depression represents an interesting results as this protein was strongly associated with inflammation and immune response, as it was shown to be abundant in the granules of neutrophiles as well as to be expressed by platelets and megakaryocytes (Valle-Jimenez et al., 2020). Moreover, the defensin family was previously associated with schizophrenia, as DEFA4 and DEFA1b were shown to be up-regulated in patients with schizophrenia compared with controls (Gardiner et al., 2013).

With regards to the other genes reporting a q-value < 0.1 COCH, and SRGAP1, to my knowledge no studies investigated their role in mental health disorders.

The gene COCH encodes for Cochlin, a protein which was shown to be expressed in the cochlea and vestibule of the inner ear, as well as in lymphoid organs such as lymph and spleen. Cochlin functions are still poorly understood, however it was shown to play a role in various pathologies of the inner ear and eye (Rhyu, Bae, Jung, & Hyun, 2020; Shiiba et al., 2012).

The SRGAP1 gene encodes for the SLIT-ROBO Rho Gtpase Activating Protein 1, which is a GTPase activator that was observed to negatively regulate neuronal migrations (Tcherkezian & Lamarche-Vane, 2007).

Females, MDD *vs* HR. In the comparison MDD *vs* HR females, only the Chorionic Somatomammotropin Hormone 2 (CSH2) gene presented a q-value < 0.1 However, to my knowledge no studies investigated its role in relation to contexts relevant to this doctoral thesis or in relation to depression. Indeed, the CSH2 protein is produced only during pregnancy and it was shown to be involved in the stimulation of lactation, fetal growth and metabolism (Handwerger & Freemark, 2000).

Females, HR *vs* LR. In this comparison, the most interesting gene with a q-value < 0.1 is the Leukocyte Immunoglobulin Like Receptor A5 (LILRA5), which was down-regulated in HR females compared with the LR counterpart (FC -1.66, q-value 0.091). The interest toward this gene is due to its major role in inflammation as it was shown to induce the production of several cytokines, such as IL-10, and its mRNA is regulated by pro-inflammatory cytokines such as TNF and IFN-γ (Mitchell, Vaze, & Rao, 2009).

Moreover, LILRA5 was shown to be a possible biomarker in predicting lithium response in males with bipolar disorder (Eugene, Masiak, & Eugene, 2018).

With regards to the other genes reporting a q-value < 0.1 TACSTD2, TBC1D3, SOX5, and DET1, to the current knowledge no studies investigated their role in mental health disorders or possibly associated with the context of this doctoral thesis.

The gene TACSTD2 encodes for the Tumor Associated Calcium Signal Transducer 2 protein, which was shown to be a carcinoma associated antigen (Linnenbach et al., 1993) and it was shown to be differently expressed in several cancers (Shvartsur & Bonavida, 2015),

The SOX5 gene encodes for the Sry-box Transcription Factor 5, which is a member of the SOX family. These proteins are a group of transcription factors deeply involved in tumorigenesis and cancer; SOX5 was shown to be involved in controlling the cell fate and differentiation in cancer (Grimm et al., 2020).

Lastly, the gene DET1 encodes for Partner Of COP1 E3 Ubiquitin Ligase and it was shown to promote ubiquitination and degradation of the protooncogenic transcription factor c-Jun (Wertz et al., 2004).

5.6 Biological pathways differently modulated between MDD and HR adolescents: focus on inflammation and immune system related pathways

The major finding from the pathways analysis performed on the genes differently expressed in the adolescents with depression compared with their peers with high risk of developing the disorder, was an enrichment in several biological signatures associated with inflammation and immune system. This finding was consistent in the analysis considering both the entire cohort and males or females separately.

When considering the comparison MDD *vs* HR in the entire cohort, several pathways were identified as being significantly modulated, and some of them were in common between the two platforms and shared common genes. Among the most interesting pathways that were identified: the role of the Hypercytokinemia/Hyperchemokinemia in the Pathogenesis of Influenza, the Interferon Signaling, the Complement System, the Activation of IRF by Cytosolic Pattern Recognition Receptors, and the Acute Phase Response Signaling. It is evident that all these pathways are associated with inflammation and immune response, suggesting a possible inflammatory landscape as underpinning the differences between the two groups. Moreover, among these pathways, the one related to the role of the Hypercytokinemia/Hyperchemokinemia in the pathogenesis of Influenza shared a positive z-score in both platforms, indicating its up-regulation in adolescents with depression compared with HR adolescents, and thus representing a common result suggesting a possible involvement of inflammation in adolescent depression.

The Interferon Signaling pathway and the Complement System pathway presented a positive z-score in the RNA-Seq results ,and the DEGs associated with these pathways and in common between the two platforms share a common direction, suggesting though a consistent possible role of both pathways as mapping the presence of adolescent depression, thus strengthening the hypothesis of a modulation of the inflammatory system in MDD compared with HR adolescents.

The link between inflammation and MDD is supported by gene expression studies on mRNA transcripts and, in line with the data shown in this doctoral thesis, an up-regulation of genes involved in the Interferon signaling pathways was observed in the central nervous system and in peripheral blood of patients with depression (Ciobanu & Baune, 2018; Jansen et al., 2016; Mostafavi et al., 2014). Previous literature observed significant association between depression and Interferon α/β signaling pathways' genes in large population-based sample (Ciobanu & Baune, 2018; Magri et al., 2021). Type I Interferons accounts for IFN-α and IFN-β, the main cytokines of the innate immune system that respond primarily to viral infection and to malignant cells. A chronic activation of this signaling cascade was shown to play a role in autoimmune and neuroinflammatory disorders. Moreover, the activation of Interferon pathway in depression is consistent also with previous literature, as it is well known that patients receiving IFN-I therapies (IFN-α for hepatitis C or IFN-β for multiple sclerosis) often develop clinically significant depression (Hepgul et al., 2016; Hepgul et al., 2018).

The complement system pathway was shown to be up-regulated in adolescents with depression compared with the HR group. The role of complement signaling was already discussed in the previous chapter, as the C4BPA gene was reported to survive the FDR correction and to be down-regulated in adolescents with depression

compared with the LR group. Although few studies investigated the role of complement in depression, given its role in activating the inflammatory response and considering the link between inflammation and depression, it is possible to suggest that its activation in adolescents with depression might be part of the general inflammatory trend suggested also by the previously discussed pathways. Interestingly, the complement system was shown to influence the Interferon signaling, as the complement component 3 was shown to mediate systemic IFN-β-induced changes in neuroinflammation and behavior of mice subjected to unpredictable chronic mild stress (Tripathi et al., 2021). Moreover, a correlation was shown also in human, as increased expression levels of IFN-I stimulated genes and significant correlation with complement 3 and inflammatory markers were observed in the prefrontal cortex of suicide subjects with depression (Tripathi et al., 2021).

Regarding the pathway of the Role of Hypercytokinemia/hyperchemokinemia in the pathogenesis of Influenza, which presented a positive z-score and thus it is suggested to be up-regulated in MDD condition, to my knowledge, no studies investigated this specific pathway in the context of mental health disorders such as depression, but they all focused on its activation in virus response and influenza. However, a great body of studies focused their attention on the role of cytokines in depression and increased levels of cytokines were widely showed in depressed patients compared with control individuals. In adulthood, higher levels of peripheral inflammatory cytokines were widely observed in patients with depression (Kohler et al., 2017; Leighton et al., 2018; Osimo et al., 2019; Osimo, Pillinger, et al., 2020). For example, increased levels of pro-inflammatory cytokines such as IL-6, IL-10, IL-12, IL-13, IL-18, and TNF-α were observed in serum and plasma levels of depressed patients compared

with healthy controls, and increased levels of IL-6 and TNF-α were observed in CSF and *post-mortem* brain tissue of adults with depression compared with healthy controls. (Enache, Pariante, & Mondelli, 2019). Moreover, the hyperactivation of the immune system were considered as a risk factor for the development of depression (Raison et al., 2006). Increased levels of pro-inflammatory cytokines were observed also in adolescent individual with depression (D'Acunto et al., 2019; Khandaker et al., 2014; G. E. Miller & Cole, 2012). The meta-analysis of D'Acunto and colleagues showed higher TNF-α levels in adolescents aged up to 18 years old with depressive disorders versus control subjects (D'Acunto et al., 2019), and higher levels of CRP and IL-6 in adolescents with depression compared with their non-depressed peers were reported in the meta-analysis of Colasanto and colleagues (Colasanto et al., 2020). Similarly to adulthood, increased cytokines levels and in general increased inflammation was related to higher risk of developing depression also in adolescents. For example, high levels of IL-6 were observed to be associated with greater depression risk (Khandaker et al., 2014), as well as increases in TNF-α were shown to predict an increase in depressive symptoms (Moriarity et al., 2020). Moreover, increased inflammation was observed to be associated with increased risk of MDD when associated with early in life trauma (Z. Zajkowska, Walsh, et al., 2021).

Thus, these results suggested that the hyperactivation and secretion of pro-inflammatory cytokines were widely associated with depression, and the up-regulation of such pro-inflammatory pathways showed in adolescents with depression compared with their HR peers is in line with the previous literature, suggesting thus a possible role of pro-inflammatory cytokines in mapping the presence of depression also in adolescents from LMICs.

Lastly, although no z-score was detected but given the previous results, it might be reasonable to believed that both the Activation of IRF by Cytosolic Pattern Recognition Receptors and the Acute Phase Response Signaling pathways could play a role in mapping adolescents with depression compared with non-depressed HR controls. Accordingly to the literature, both pathways plays an active role in inflammatory response as well as in depression. For instance, proteins involved in the acute phase response were shown to be up-regulated in MDD patients compared with controls (Q. Wang et al., 2019). One of the most studied among the acute phase proteins is the C-Reactive Protein (CRP), which is induced by the IL-6 action on the gene responsible for the transcription of CRP during the acute phase of an inflammatory process (Gabay & Kushner, 1999). CRP was shown to play a role in the recognition of pathogens and damaged cells and in activating the component pathways (Black, Kushner, & Samols, 2004). Peripheral blood CRP levels was also widely used to measure systemic inflammation, and different levels of CRP were established and widely used also in clinical trials using anti-cytokines therapies for MDD, in order to select depressed patients based on elevated levels of peripheral blood CRP concentrations (A. H. Miller, Haroon, & Felger, 2017). Moreover, CRP levels were also associated with resistance to antidepressant treatments, as elevated blood levels of CRP were observed in TRD patients with depression compared with both treatment-responsive and untreated patients (Chamberlain et al., 2019). As mentioned also in the studies cited before, increased levels of CRP were showed in blood of adult individuals with depression as well as in adolescents (Felger et al., 2020; Howren, Lamkin, & Suls, 2009; Jung & Kang, 2019), and increased levels of CRP were also associated with increased risk of developing depression (Osimo, Stochl, et al., 2020). Consequently, given the evidence

of increased CRP levels in association with depression, the identification of a possible up-regulation of the Acute Phase Response Signaling pathway in MDD *vs* HR adolescents showed in this doctoral thesis is in line with the literature, suggesting thus a possible role of inflammation and immune system as biological signatures characterizing adolescent depression in this Brazilian cohort of adolescents.

5.6.1 The role of biological sex on the inflammatory pathways activated in MDD compared with HR adolescents

The reason behind investigating both genes differentially expressed and the related pathways by considering males and females separately lies in the well-known difference in the incidence of depression in males and females. Therefore, I hypothesized that differences in the pathways underlying the presence of depression might be associated with biological sex.

The results of the pathways analysis from the comparison MDD *vs* HR in both males and females showed interesting and unexpected results, together with discrepancies among the two different techniques used.

The pathways analysis in females with depression compared with the HR counterpart showed very similar results to what I observed in the entire cohort. Among the pathways observed to be significantly modulated in females only, four of them were also observed in the analysis in the entire cohort previously described as well in common between the two -omics approaches; specifically they are: the Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza, the Activation of IRF by Cytosolic Pattern Recognition Receptors, the Interferon Signaling, and the Acute Phase Response Signaling. As discussed in the previous section, all these

pathways seem to be connected with inflammation, and their positive modulation in females with depression compared with HR females is consistent with the results observed for the entire cohort. Thus, these results might suggest that females with depression presented an up-regulation of pathways associated with inflammation, and this is in line with previous studies in literature showing increased levels of inflammation and pro-inflammatory cytokines in females with depression compared with control female individuals (Henje Blom et al., 2012; Pallavi et al., 2015).

On the other hand, the results in males are of more difficult interpretation given the fact that the two platforms showed conflicting results. The pathways analysis on DEGs from microarrays show a very robust modulation towards activation of inflammatory and immune system mechanisms in males with depression compared with HR peers, thus showing consistent results with the entire cohort. In contrast, no such inflammatory pattern resulted from the DEGs from RNA-Seq: the pathways resulted to be differently modulated in males are mostly related to cell-cycle and presented a very heterogenous biological profile. These discrepancies are likely attributable to the intrinsic differences between microarray and RNA-Seq, as previously described in the paragraph 5.3. Thus, it is possible that the inflammatory activation in males with depression shown by Affymetrix results might be less prominent than the one observed in females, and thus it was not replicated in the RNA-Seq data.

Overall, differently to what initially hypothesized, no specific or uniquely sex-related pathways associated with depression were shown in males or females separately compared with the entire cohort. However, the consistent modulation of inflammatory pathways in females and the inconsistent results in males might suggest

that the modulation of such inflammatory and immune system related pathways observed in the entire cohort could be driven by females.

To conclude, a modulation towards activation of inflammation-related pathways in adolescents with depression compared with their non-depressed HR peers, represents an important finding of this doctoral thesis. It is noteworthy to remember that also adolescents with depression were identified as having high risk of developing the disorder accordingly to the risk score (Kieling et al., 2021), thus the only difference between the MDD and HR group is the current diagnosis of depression for the former. This represents an important point as it may suggest that inflammation might be able to map the presence of depression in adolescents classified as having high risk of developing the disorder.

Acknowledging the current literature, the role of inflammation in the pathophysiology of depression in adolescents was less studied than in adults, despite the fact that adolescence represent a critical temporal window for both the onset of depression as well as for the major changes faced by the immune system, including reduction in lymphatic tissue size and changes in sex hormones that affect the release of cytokines (Osimo, Stochl, et al., 2020; Toenders et al., 2021). Nevertheless, the general pattern of modulation of inflammation in adolescents with depression showed in this doctoral thesis is consistent with most studies conducted so far investigating the role of inflammation in adolescent depression. Indeed, as previously shown, increased levels of pro-inflammatory cytokines were observed in adolescents with depression compared with controls (Gabbay, Klein, Alonso, et al., 2009; Gabbay, Klein, Guttman, et al., 2009; Kautz et al., 2020; Khandaker et al., 2014; Rengasamy et al., 2021). On the

other hand, further studies did not find any correlation between inflammation and depression in adolescents (Chaiton, O'Loughlin, Karp, & Lambert, 2010; McDade, Borja, Adair, & Kuzawa, 2013; Reid et al., 2020).

However, given the fact that the results of this doctoral thesis are part of the IDEA project which aims to identify pathways associated with adolescent depression in LMICs (as Nepal and Nigeria), it is noteworthy to acknowledge and discuss that very few studies were so far conducted in LMICs. In the study of Perez-Sanchez investigating depression in a cohort of 14-19-years-old in Mexico City, increased levels of pro-inflammatory cytokines were observed in depressed individuals compared with controls (Perez-Sanchez et al., 2018). In contrast, no increased inflammation was shown in two different studies investigating community-based adolescent depression in Philippines and Chile (McDade et al., 2013; Reid et al., 2020). An interesting explanation of such results, which are opposite to most of other studies conducted in HICs, was that chronic inflammation levels might be lower in environment characterized by higher prevalence of infection disease, such as the Philippines (McDade et al., 2013).

Hence, the results of this doctoral thesis might suggest that inflammation could be considered as a biological pathways mapping depression in those adolescents who were already classified as having high risk of developing the disorder accordingly to the risk score. It is important to acknowledge that the risk score was based on sociodemographic variables that in turn accounted for diverse environmental risk factors, so it is reasonable to believe that the association of both environmental – such

as childhood trauma or parental relationship - and biological risk factors – such as inflammation, can be suggested to play a common role in the risk of developing depression, and that can in turn be used for the prediction of the risk of developing adolescent depression. This is in line with the results of the systematic review conducted in the context of the IDEA project by our group, which showed an increased association of inflammation with the onset of MDD in adolescence, but particularly in the context of early life stress (Z. Zajkowska, Walsh, et al., 2021). The link between early in life stress and biological changes leading to vulnerability to develope MDD in adults was widely reported (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016; Heim & Nemeroff, 2001), and a mediation model was proposed to explain these findings, suggesting the hyperactivation of the sympathetic nervous system as the mediator between the experience of early in life stress and the activation of the inflammatory system which leads to the onset of depression (V. Mondelli & Vernon, 2019). However, this association was also explained by a moderation model, as Miller and colleagues showed that higher experiences of childhood trauma were associated with higher inflammatory levels in individual with depression, suggesting thus that increased inflammation was only associated with depression in adolescents exposed to childhood adversity (G. E. Miller & Cole, 2012). Therefore, it is reasonable to believe that considering multiple risk factors, such as environmental risk factors (e.g., childhood trauma) and biological markers (e.g., inflammation) might provide a higher predictive power as well as might help in the development of prevention strategies to modify trajectories from the experience of early adversities to the development of depression during adolescence.

5.7 Biological differences between MDD and LR adolescents

One of the innovative aspects of using the IDEA-RiSCo cohort is that it does not assume that adolescents without depression constitute a homogenous group. Most of the currently available mental health samples compare adolescents with depression *vs* adolescents without depression. However, adolescents without depression may have several risk factors that make them likely to develop a disorder in the future, leading to a high heterogeneity of findings in these studies. Comparing adolescent with depression separately with HR adolescents and then with LR adolescents allows us to identify potential pathways linked to development of depression *vs* pathways associated with presence of risk factors.

The biological pathways analysis performed on the DEGs from the comparison of adolescents with depression and LR adolescents in the entire cohort, showed the modulation of pathways associated with inflammation: the role of the Hypercytokinemia/hyperchemokinemia in the pathogenesis of Influenza, the Interferon Signaling, the Osteoarthritis Pathways, and the Complement System. The first three pathways were reported to have a positive z-score accordingly to RNA-Seq, indicating their up-regulation in adolescents with depression compared with the LR group.

The role of the Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza, the Interferon Signaling, and the Complement System pathways were already observed to be activated also in adolescents with depression compared with the HR. An enhance activation of inflammation in adolescents with depression also when compared with LR strengthens the hypothesis that inflammation could map the presence of depression in adolescents

Moreover, in the pathways analysis resulted from RNA-seq, it is interesting to notice that the Calcium Signaling and the Glutamate Receptor Signaling pathways were down-regulated in adolescents with depression. Both these pathways were previously associated with stress-related disorders and depression (Deutschenbaur et al., 2016). Calcium signaling pathways, together with the MAPK signaling, were observed to be down-regulated in the nucleus accumbens of rats exposed to chronic-mild stress and that develop a depressive like-behavior, whereas the up-regulation of this pathway was shown to be associated with resilience to depression (Si, Song, Sun, & Wang, 2018). Thus, the down-regulation of this pathways in adolescents with depression shown in this doctoral thesis is in line with the literature's results.

Alterations in the glutamatergic neurotransmission were previously observed in depression (Niciu, Ionescu, Richards, & Zarate, 2014), and alteration of glutamate levels in brain regions of patients with depression were observed by using magnetic resonance spectroscopy (MRS), although heterogenous findings were observed, as both an increase in brain glutamate in the basal ganglia as well as a decrease in glutamate levels were reported in depressed patients (Haroon & Miller, 2017; Kondo et al., 2011; Yuksel & Ongur, 2010). Indeed, the role of glutamate in depression was also shown by the antidepressant effects of the glutamatergic receptor NMDA antagonist Ketamine (Nikkheslat, 2021). Interestingly, inflammation was proposed as a possible mechanism responsible for the alterations of the glutamate system in depression (Haroon & Miller, 2017). Specifically, inflammatory process were shown to impact several aspects of glutamate neurotransmission, including alterations in the glutamate release and re-uptake mechanisms, and activation of metabolic pathways

involving the enzyme IDO and the KYN pathway, that can produce neuroactive metabolites which can in turn affect glutamate metabolism (Haroon & Miller, 2017). A correlation between inflammation and the KYN pathways was observed also in the study of Haroon and colleagues, showing that individuals with depression and increased levels of plasma inflammatory markers and KYN metabolites showed an increase in depressive symptom severity, anhedonia, and treatment non-responsiveness (Haroon et al., 2020). To note, ~~i~~t is not unusual to find such signaling pathways as differently modulated in peripheral tissue such as blood, as 18 known subunits of the ionotropic glutamate receptors were found to be expressed in human peripheral blood mononuclear cells (Bhandage et al., 2017).

Thus, it is possible to suggest that impairment in the glutamate transmission might represent an important difference mediating the biological differences between HR adolescent with a current diagnosis of depression and their peers with a low risk of developing depression. My data are in line with the current literature suggesting that an imbalance in the glutamatergic transmission together with an increased activation of inflammation might have a role in adolescent depression (Haroon & Miller, 2017; Haroon et al., 2020; Kadriu et al., 2019; Sanacora, Treccani, & Popoli, 2012).

5.7.1 The role of biological sex on the pathways differently modulated in MDD compared with LR adolescents

When considering males and females separately, the results presented some discrepancies when comparing the two -omics approaches. Affymetrix pathways analysis showed the up-regulation of signatures associated with inflammation in both males and females with depression, whereas the biological signatures resulted from

RNA-Seq analysis did show a more heterogenous picture of pathways in males and females with depression. Specifically, in the MDD vs LR comparison in males from the RNA-Seq, most of the pathways differently regulated were mainly associated with the cell cycle. On the other hand, the same comparison in females resulted in some pathways related to inflammation, although the z-score direction was more heterogeneous. For example, I observed a negative z-score in the STAT3 pathways, CDK5 signaling, and PAK Signaling, indicating their down-regulation in females with depression compared with LR; on the other hand, a positive z-score resulted for the Phagosome formation and Natural Killer Cell Signaling pathways. All these pathways were shown to be associated with inflammation, thus their different modulation contributed to the lack of a general trend toward an up-regulation or down-regulation of inflammatory related pathways as I observed in the MDD *vs* HR comparison. These discrepancies might be due indeed to the different -omics techniques which were used, and thus to the different DEGs detected by the two platforms. However, these differences might strengthen the hypothesis that inflammation might map the presence of depression mostly when comparing depressed and non-depressed adolescents both classified as having high risk of developing MDD.

5.8 Biological signatures associated with the risk of developing depression in adolescents without depression (HR vs LR)

As previously described, the IDEA-RS was developed based on eleven sociodemographic variables to distinguish between those adolescents with higher risk of developing depression and those with a lower risk. The diagnosis of depression was assessed after the risk score assessments, that is why all the adolescents with depression are also classified as having high risk of developing MDD. Thus, HR and LR adolescents represent in a way the control individuals with different risks of developing MDD accordingly to sociodemographic variables. One of the aims of this doctoral thesis was to investigate possible biological pathways associated with a higher or lower risk of developing depression, to possibly pave the way to preventive strategies as well as better dissecting vulnerability and resilience signatures in adolescents.

The results from the pathways analysis showed very heterogenous results, meaning that not a specific unique biological pattern of signatures was identified to be differently modulated between HR and LR. These results might also be related to the very low correlation between DEGs resulted from the comparison of the two techniques. Indeed, differently from the good correlation observed in the comparison MDD *vs* HR and MDD *vs* LR, for the HR *vs* LR comparison the Person's correlation coefficient was low even when considering the common genes most significantly modulated. This might suggest that the biological differences between non-depressed adolescents classified for different degrees of risk for depression could be less evident and detectable, as it will be discussed at the end of this paragraph.

The pathways resulted from the microarray data did not present a positive or negative z-score, both when considering the entire cohort or males and females separately. When considering the pathways resulted from the entire cohort, all the signatures are related to the degradation of neurotransmitters, such as Melatonin degradation, Tryptophan degradation, Noradrenaline and Adrenaline degradation, Serotonin degradation. However, given the absence of a z-score and the fact that all those pathways were identified by IPA as associated with a single differently expressed gene (SMOX), any conclusion may be too speculative. Similarly, no positive or negative z-score were identified also when looking at males and females separately.

When looking at the pathways analysis resulted from RNA Sequencing, in the entire cohort most pathways are down-regulated in HR adolescents compared with the LR group. The functional profile of these pathways is extremely heterogenous as they covered very different molecular mechanisms, such as cell communication and cardiovascular related pathways.

On the other hand, interestingly the same comparison in females resulted in some pathways ascribable to inflammation, although the z-score direction was more heterogeneous. For example, I observed a negative z-score in the Inflammasome pathways and in TREM1 Signaling indicating their down-regulation in HR females compared with LR, whereas a positive z-score resulted for STAT3 Pathways; lastly, IL-10 Signaling was identified as associated with the DEGs but the direction of the modulation (z-score) was not detected. The Inflammasome pathways (Strowig, Henao-Mejia, Elinav, & Flavell, 2012), TREM1 (DiSabato et al., 2021) and STAT3 pathways (Kong et al., 2015) were shown to be associated with inflammation, thus

their different modulation contributed to the lack of a general trend toward an activation or inactivation of inflammatory related pathways.

Lastly, in the males' analysis, the results were still very heterogenous. However, I observed a small up-regulation of the Leukocyte extravasation signaling in HR males compared with the LR counterpart, indicating thus a minor modulation of inflammation in HR males compared with females.

To conclude, it seems that the differences between HR and LR did not follow a unique and evident pattern as observed for the other comparisons, indicating thus that my analysis did not pinpoint strong and robust biological differences accordingly only to the risk of developing depression. This does not mean that the risk score is not able to detect biological differences based on the sociodemographic variables, rather that this might partly reflect the heterogeneity of the IDEA-RS, as this risk score included eleven different sociodemographic variables and different environmental risk factors. Similarly to the results found in this doctoral thesis, no differences in cytokines levels in plasma were detected between the same HR and LR groups (data submitted for publication, part of the IDEA project), whereas differences were observed in the levels of some cytokines in adolescents with depression compared with the risk groups. Nevertheless, functional MRI analysis conducted in the same IDEA cohort showed differences between HR and LR adolescents (Yoon et al., 2021). In this study, whole brain analysis found reduced reward-related activity in the lateral prefrontal cortex of patients and HR adolescents compared with LR adolescents. Compared with LR adolescents, HR and adolescents with depression showed reduced threat-related left amygdala connectivity with thalamus, superior temporal gyrus, inferior parietal gyrus,

precentral gyrus, and supplementary motor area (Yoon et al., 2021). Thus, these diverse results could suggest that different approaches for investigating the biological differences between HR and LR might be implemented and can represent a future direction, even considering the heterogeneity of the IDEA-RS.

5.9 Strength and Limitations of this doctoral thesis

One strength of this doctoral thesis is indeed the uniqueness of the IDEA-RiSCo. This cohort does not assume that adolescents without depression constitute a homogenous group; therefore, using a risk score to stratify for risk of future depression allowed the recruitment of two groups of adolescents without depression with different risk of developing depression, as these adolescents may have several risk factors that make them more or less likely to develop the disorder in the future. Indeed, this represent a unique strength, since most of the currently available samples from patients with mental disorders typically compare cases and non-cases, where the latter are defined only by lack of a current psychiatric disorder but not also for a possible risk of developing the disorder in the future. This allowed a more thorough investigation of biological mechanisms associated with depression which may depend or not on sociodemographic risk factors.

A second strength is represented by the fact that this doctoral thesis, as part of the IDEA project, aims to focus also on adolescents living in LMICs, who account for about 90% of adolescents worldwide. Indeed, the approach adopted for investigating adolescent depression in Brazil was aimed to develop strategies to be implemented and used also in LMICs which are part of the IDEA project, such as Nepal and Nigeria. This represents a strength as most of the research on depression during adolescence was conducted in cohorts from Europe and USA, highlighting a significant gap in the knowledge of biological signatures that could help in explaining how depression develops in adolescents in LMICs, where the psychosocial environment often differs from HICs.

A third strength is represented by the hypothesis-free approach used for the investigation of the biological mechanisms underlying the presence of depression and the different risk of developing it. The use of a genome-wide gene expression approaches allowed the study of such biological pathways by investigating the entire transcriptome, without *a priori* biased hypothesis and allowing thus to identify biological signatures or pattern never studied before.

However, this doctoral thesis has also some limitations. Firstly, the genome-wide gene expression techniques used are different and differences in terms of genes differently expressed were observed. Although a good correlation was observed when considering the DEGs most significantly modulated in both the two platforms, differences in terms of DEGs were observed, together with a very small number of genes surviving the FDR correction. For these reasons, it was not possible to center the main focus of the investigation and of the discussion on specific DEGs, but the attention was rather aimed on the biological pathways differently modulated in each comparison. The results in terms of both DEGs and pathways analysis, together with the advantages and disadvantages of both techniques that were previously explained in the discussion section, lead us to consider the RNA-Seq as the most valid -omic technique to be used for future studies, such as the 3-years follow up of the present IDEA study.

Secondly, this is a cross-sectional study, so to date it is not possible to suggest whether the biological signatures identified in the HR group will predict the development of depression later during adolescence. Consequently, it would be important to follow-up the participants to understand whether HR adolescents will develop depression or

not and therefore to identify vulnerability mechanisms and related biomarkers associated with the risk of developing MDD. To this point, it is possible that some adolescents in the HR group will be resilient and will not develop depression, as it is well-known that individuals exposed to risk for developing depression – such as early in life stress- might not end up in developing the disorder. Therefore, identifying adolescents who are resilient to depression even though classified as having a high risk, might represent an important starting point for investigating the biological pathways underlying resilience to depression. However, this limitation have already been acknowledge as a follow-up study, called IDEA-Flame, is ongoing (Valeria Mondelli et al., 2021).In the IDEA Flame study, the same adolescents are undergoing a three-years follow-up, and the blood sample collection as well as the clinical evaluation is currently ongoing. We plan to perform RNA-Seq analysis on blood samples, to identify possible biological mechanisms that lead adolescents from a condition of risk to the current presence of MDD. Moreover, we will be able to identify those adolescents who did or did not developed depression accordingly to their risk score, and thus investigate biological mechanisms associated with vulnerability and resilience to depression.

A third limitation is represented by the IDEA risk score. Specifically, although it was validated to predict depression (Rocha et al., 2021), it was also shown to predict the development of other psychiatric disorders, albeit with somewhat lower accuracy. For this reason, future longitudinal studies are needed to examine whether the observed biological signatures 1) predict the development of depression, 2) whether their predictive accuracy is greater for depression than other types of mental disorders, and

3) whether there are any differences in the observed biological signatures in earlier onset versus later-onset MDD.

Lastly, the IDEA risk-score was validated in HIC setting as well as in LMIC countries such as Nepal and Nigeria. The validation shown good discriminative values in both settings; however, adjustments were made in order to apply the risk factors in such different settings. This might represent a limitation as the IDEA-RS must be adjust accordingly to the different cultural, economic, and social background of the populations.

5.10 Identifying biological signatures underlying the presence of depression or the risk of developing the disorder – Clinical implications and future directions.

In my doctoral thesis, I identified that adolescents with depression showed an enrichment in inflammation and immune system related pathways compared with adolescents without depression. Furthermore, the results might also suggest that biological sex could play a role in modulating the pathways differently regulated among adolescents with and without depression, and specifically the modulation of inflammatory pathways seems to be more pronounced in females rather than in males. Lastly, I did not identify a panel of pathways differently modulated between HR and LR adolescents without a diagnosis of depression.

This doctoral thesis has the advantage of leveraging on a unique risk-stratified cohort of adolescents and of addressing the issues of comparing adolescents with depression with a potentially widely heterogenous sample of controls but stratified for risk of developing the depression accordingly to sociodemographic variables. Understanding the biological signatures involved in the presence or in the risk of adolescent depression will help to better identify adolescents at higher risk of developing depression at an early stage and potentially act before the onset of the symptomatology, by pinpointing potential key therapeutic targets to prevent the onset of adolescent depression worldwide. In this context, a longitudinal follow-up is needed to specifically identify mechanisms and trajectories leading from a risk of developing depression to the actual presence of the disorder. However, considering the follow-up study that is currently ongoing, it is reasonable to hypothesize that the

identification of differently regulated biological pathways might be useful in order to improve the identification of adolescents at risk at an early stage, to support targeted prevention interventions, and to better treat adolescent depression.

These steps are extremely important to improve the knowledge of adolescent depression and contribute to the development of effective early interventions that would potentially reduce the burden of depression in adolescence.

5.11 Conclusions

To conclude, I identified inflammation and immune system as leading biological pathways mapping the presence of adolescent depression in a cohort of 14-16 years-old in Brazil. The modulation toward the up-regulation of inflammatory and immune system pathways in adolescents with depression resulted to be more evident when comparing adolescents with depression with the HR group, and this might represent an important observation as both depressed and HR adolescents are classified at high risk of developing MDD accordingly to the IDEA-RS. Therefore, at this stage an enhanced activity of inflammation might represent the biological pathways mapping the presence of depression in adolescents. Further investigations, and specifically a longitudinal study, will be required to understand whether HR adolescent who developed depression at the three-years follow-up showed an enhanced modulation of inflammatory pathways at baseline, as well as understanding whether the absence of inflammation might represent a resilience mechanism for those adolescents who did not develop depression at the follow-up (Valeria Mondelli et al., 2021).

On the other hand, I did not find a homogeneous panel of biological pathways able to distinguish between adolescents without depression accordingly to the risk group. This result might partly reflect the heterogeneity of the IDEA-RS, as this risk score included diverse sociodemographic variables and different environmental risk factors, suggesting thus that the lack biological differences between HR and LR adolescents might reflect the heterogeneity of the sociodemographic and environmental variables considered by the IDEA-RS.

Lastly, given the differences in the results from the two -omics techniques applied and considering the numerous limitations of the microarrays, RNA-Seq was proved to be

more sensitive in terms of genes differently expressed in each comparison as well as in terms of pathways analysis. For this reason, the -omics analysis in the follow-up study will be performing exclusively by using the RNA-Seq technique (Mondelli et al., 2021).

Overall, the findings resulted in this doctoral thesis can represent a step toward the untangling of the complex system involved in the physiopathology of adolescent depression as well as in the risk of developing the disorder. Further studies, such as the ongoing longitudinal follow-up, might represent an important step toward the identification of mechanisms of vulnerability and resilience, and thus toward the ambitious goal of reducing the burden associated with adolescent depression specifically in the poorest settings, and that can be translated in the clinical practice worldwide.

*6. **References***

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APPENDIX A

Table A. RNA concentration, absorbance values at 260 nm and 280 nm, and A260/280 and A260/230 ratios. *For the risk group: A-High Risk; B-Low Risk; C-MDD.*

Sample ID	Risk group	Nucleic Acid Concentration	Unit	A260	A280	260/280	260/230
BR0001PAX	C	179.6	ng/µl	4.49	2.147	2.09	1.85
BR0002PAX	C	280.6	ng/µl	7.015	3.363	2.09	0.96
BR0005PAX	A	166.6	ng/µl	4.164	1.994	2.09	1.46
BR0006PAX	C	380.5	ng/µl	9.512	4.557	2.09	1.51
BR0009PAX	C	53.9	ng/µl	1.348	0.672	2.01	1.83
BR0013PAX	B	232.4	ng/µl	5.811	2.767	2.1	1.89
BR0015PAX	C	183	ng/µl	4.575	2.234	2.05	1.18
BR0018PAX	C	121.2	ng/µl	3.029	1.447	2.09	0.32
BR0019PAX	C	154.5	ng/µl	3.862	1.873	2.06	0.68
BR0020PAX	A	109.9	ng/µl	2.747	1.321	2.08	1.54
BR0021PAX	C	196.2	ng/µl	4.904	2.381	2.06	1.74
BR0022PAX	C	177.6	ng/µl	4.44	2.166	2.05	0.81
BR0024PAX	C	115.6	ng/µl	2.891	1.43	2.02	0.95
BR0027PAX	C	230.3	ng/µl	5.757	2.831	2.03	1.6
BR0028PAX	C	236.8	ng/µl	5.92	2.851	2.08	1.82
BR0029PAX	C	103.6	ng/µl	2.59	1.268	2.04	1.01
BR0030PAX	C	217.3	ng/µl	5.433	2.624	2.07	0.63
BR0032PAX	C	171.1	ng/µl	4.277	2.08	2.06	0.77
BR0033PAX	A	200.2	ng/µl	5.005	2.419	2.07	1.6
BR0035PAX	B	115.7	ng/µl	2.892	1.406	2.06	1.26
BR0036PAX	A	180.2	ng/µl	4.506	2.272	1.98	2.14
BR0037PAX	A	142.3	ng/µl	3.557	1.712	2.08	0.9

BR0038PAX	C	229.6	ng/µl	5.74	2.761	2.08	1.54
BR0039PAX	C	241.4	ng/µl	6.034	2.869	2.1	1.14
BR0040PAX	C	363.5	ng/µl	9.088	4.334	2.1	0.99
BR0043PAX	C	135.6	ng/µl	3.391	1.674	2.03	0.83
BR0044PAX	C	330.7	ng/µl	8.267	4.062	2.04	2.05
BR0045PAX	C	147.5	ng/µl	3.688	1.787	2.06	1.9
BR0046PAX	C	180.8	ng/µl	4.52	2.147	2.11	2.09
BR0047PAX	A	210.8	ng/µl	5.27	2.542	2.07	1.52
BR0048PAX	A	169.7	ng/µl	4.242	2.02	2.1	1.96
BR0049PAX	A	254.3	ng/µl	6.356	3.034	2.1	1.85
BR0050PAX	B	229.7	ng/µl	5.742	2.912	1.97	1.38
BR0051PAX	B	170	ng/µl	4.249	2.04	2.08	1.58
BR0052PAX	B	242	ng/µl	6.049	2.895	2.09	1.38
BR0053PAX	B	112.3	ng/µl	2.809	1.386	2.03	0.35
BR0054PAX	B	226.2	ng/µl	5.656	2.727	2.07	1.85
BR0055PAX	C	120.1	ng/µl	3.002	1.504	2	0.86
BR0056PAX	C	115	ng/µl	2.876	1.415	2.03	1.37
BR0057PAX	C	162.3	ng/µl	4.057	1.945	2.09	0.87
BR0058PAX	C	87	ng/µl	2.176	1.049	2.07	1.6
BR0059PAX	B	155.1	ng/µl	3.878	1.857	2.09	1.11
BR0060PAX	A	172.4	ng/µl	4.311	2.072	2.08	1.38
BR0061PAX	C	155.2	ng/µl	3.879	1.905	2.04	1.46
BR0064PAX	C	171.4	ng/µl	4.285	2.049	2.09	1.28
BR0065PAX	C	278.8	ng/µl	6.97	3.365	2.07	2.04
BR0066PAX	A	149	ng/µl	3.725	1.791	2.08	1.81
BR0067PAX	B	191.6	ng/µl	4.791	2.34	2.05	1.02
BR0069PAX	A	166.7	ng/µl	4.168	2.069	2.01	1.75

BR0070PAX	A	238.7	ng/µl	5.967	2.86	2.09	1.85
BR0071PAX	A	291.4	ng/µl	7.285	3.463	2.1	2.09
BR0072PAX	A	140.9	ng/µl	3.522	1.726	2.04	0.41
BR0073PAX	C	200.9	ng/µl	5.023	2.382	2.11	2.02
BR0074PAX	A	270.4	ng/µl	6.76	3.406	1.98	1.28
BR0075PAX	A	193.4	ng/µl	4.835	2.344	2.06	1.76
BR0076PAX	B	180.7	ng/µl	4.518	2.167	2.09	1.05
BR0077PAX	A	167.6	ng/µl	4.19	2.032	2.06	0.77
BR0078PAX	B	251.9	ng/µl	6.298	3.116	2.02	0.97
BR0079PAX	B	191.2	ng/µl	4.78	2.296	2.08	1.5
BR0080PAX	B	198.1	ng/µl	4.952	2.359	2.1	0.63
BR0081PAX	B	117.3	ng/µl	2.932	1.406	2.09	2.09
BR0082PAX	B	193.3	ng/µl	4.833	2.316	2.09	1.28
BR0083PAX	C	230	ng/µl	5.751	2.768	2.08	0.7
BR0084PAX	B	157.6	ng/µl	3.94	1.99	1.98	0.89
BR0085PAX	B	247.3	ng/µl	6.182	2.935	2.11	0.98
BR0086PAX	A	160.3	ng/µl	4.007	1.912	2.1	1.09
BR0087PAX	B	180.7	ng/µl	4.519	2.191	2.06	1.71
BR0088PAX	A	135	ng/µl	3.375	1.622	2.08	1.09
BR0089PAX	A	210.5	ng/µl	5.262	2.519	2.09	1.35
BR0091PAX	C	181.7	ng/µl	4.543	2.205	2.06	1.38
BR0092PAX	A	171.8	ng/µl	4.296	2.051	2.09	0.37
BR0094PAX	C	175.7	ng/µl	4.394	2.255	1.95	1.4
BR0095PAX	A	198.9	ng/µl	4.972	2.437	2.04	1.25
BR0096PAX	A	125.9	ng/µl	3.147	1.512	2.08	1.25
BR0097PAX	A	215.1	ng/µl	5.378	2.788	1.93	1.28
BR0099PAX	B	242	ng/µl	6.051	2.876	2.1	0.59

BR0100PAX	B	252.6	ng/µl	6.314	3.024	2.09	0.86
BR0101PAX	A	179.8	ng/µl	4.494	2.274	1.98	1.03
BR0103PAX	C	230	ng/µl	5.751	2.772	2.07	1.59
BR0104PAX	A	268.7	ng/µl	6.717	3.241	2.07	1.45
BR0105PAX	B	111.1	ng/µl	2.778	1.345	2.07	1.69
BR0106PAX	B	314.8	ng/µl	7.87	3.746	2.1	1.86
BR0107PAX	A	290.2	ng/µl	7.254	3.49	2.08	1.49
BR0108PAX	A	208.5	ng/µl	5.213	2.484	2.1	1.94
BR0109PAX	A	268.7	ng/µl	6.718	3.239	2.07	2.01
BR0110PAX	A	132.7	ng/µl	3.317	1.68	1.97	1.16
BR0111PAX	A	150.3	ng/µl	3.758	1.833	2.05	1.68
BR0112PAX	A	157.9	ng/µl	3.948	1.992	1.98	1.45
BR0113PAX	B	222.8	ng/µl	5.569	2.798	1.99	1.63
BR0114PAX	B	138.1	ng/µl	3.452	1.71	2.02	1.31
BR0115PAX	B	126.6	ng/µl	3.164	1.532	2.07	1.53
BR0117PAX	B	145.6	ng/µl	3.64	1.746	2.09	0.97
BR0120PAX	B	110.6	ng/µl	2.766	1.352	2.05	0.8
BR0121PAX	A	215.4	ng/µl	5.385	2.586	2.08	0.52
BR0122PAX	C	224.6	ng/µl	5.615	2.788	2.01	1.03
BR0128PAX	B	221.7	ng/µl	5.543	2.683	2.07	1.64
BR0131PAX	B	197.2	ng/µl	4.929	2.337	2.11	0.51
BR0137PAX	B	107.6	ng/µl	2.691	1.286	2.09	1.38
BR0140PAX	A	121.2	ng/µl	3.029	1.455	2.08	0.46
BR0142PAX	B	147.6	ng/µl	3.69	1.799	2.05	1.66
BR0143PAX	A	206.8	ng/µl	5.171	2.467	2.1	1.59
BR0144PAX	B	146.1	ng/µl	3.652	1.74	2.1	1.15
BR0151PAX	B	252.6	ng/µl	6.316	3.024	2.09	1.33

BR0152PAX	B	168.3	ng/µl	4.206	1.996	2.11	1.07
BR0157PAX	B	77.6	ng/µl	1.94	0.934	2.08	0.29
BR0090PAX	C	254.8	ng/µl	6.37	3.108	2.05	2.18
BR0093PAX	A	193.67	ng/µl	4.842	2.362	2.05	2.28
BR0098PAX	A	148.58	ng/µl	3.714	1.815	2.05	0.61
BR0102PAX	A	120.32	ng/µl	3.008	1.483	2.03	1.4
BR0116PAX	A	144.47	ng/µl	3.612	1.783	2.03	2.19
BR0118PAX	A	152.58	ng/µl	3.815	1.866	2.04	1.01
BR0119PAX	A	159.49	ng/µl	3.987	1.96	2.03	1.83
BR0123PAX	C	58.61	ng/µl	1.465	0.725	2.02	1.85
BR0124PAX	C	121.91	ng/µl	3.048	1.507	2.02	1.48
BR0125PAX	A	96.61	ng/µl	2.415	1.19	2.03	2.16
BR0127PAX	C	180.02	ng/µl	4.5	2.215	2.03	0.92
BR0129PAX	A	110.04	ng/µl	2.751	1.349	2.04	1.99
BR0130PAX	A	62.99	ng/µl	1.575	0.778	2.02	0.58
BR0132PAX	C	188.97	ng/µl	4.724	2.341	2.02	2.18
BR0133PAX	B	103.21	ng/µl	2.58	1.278	2.02	1.28
BR0134PAX	C	199.91	ng/µl	4.998	2.48	2.02	1.59
BR0135PAX	B	197.89	ng/µl	4.947	2.421	2.04	2.19
BR0136PAX	B	122.97	ng/µl	3.074	1.522	2.02	2.12
BR0138PAX	A	82.95	ng/µl	2.074	1.014	2.05	2.21
BR0139PAX	B	105.49	ng/µl	2.637	1.309	2.01	1.71
BR0141PAX	B	211.94	ng/µl	5.299	2.658	1.99	1.71
BR0145PAX	B	97.95	ng/µl	2.449	1.218	2.01	0.74
BR0146PAX	A	168.87	ng/µl	4.222	2.071	2.04	2.13
BR0147PAX	B	127.19	ng/µl	3.18	1.59	2	2.33
BR0148PAX	B	156.98	ng/µl	3.925	1.944	2.02	2.08

BR0149PAX	A	91.31	ng/µl	2.283	1.109	2.06	1.41
BR0150PAX	A	227.14	ng/µl	5.679	2.795	2.03	2.06
BR0153PAX	B	168.7	ng/µl	4.217	2.07	2.04	2.13
BR0154PAX	A	151.01	ng/µl	3.775	1.837	2.05	2.2
BR0155PAX	A	91.88	ng/µl	2.297	1.118	2.05	2.24
BR0156PAX	C	140.47	ng/µl	3.512	1.726	2.04	2.12
BR0158PAX	B	80.77	ng/µl	2.019	0.985	2.05	2.17
BR0160PAX	B	81.81	ng/µl	2.045	1.004	2.04	1.84
BR0161PAX	B	90.9	ng/µl	2.272	1.117	2.03	1.55
BR0162PAX	B	170.03	ng/µl	4.251	2.107	2.02	1.41
BR0163PAX	C	175.13	ng/µl	4.378	2.216	1.98	2.17
BR0164PAX	B	186.94	ng/µl	4.673	2.293	2.04	2.31
BR0165PAX	C	128.43	ng/µl	3.211	1.575	2.04	2.19
BR0167PAX	C	206.16	ng/µl	5.154	2.521	2.04	2.22
BR0168PAX	B	109.09	ng/µl	2.727	1.333	2.05	1.71
BR0169PAX	C	137.64	ng/µl	3.441	1.683	2.04	1.32
BR0170PAX	C	323.53	ng/µl	8.088	4.012	2.02	2.2
BR0171PAX	C	268.63	ng/µl	6.716	3.28	2.05	2.07
BR0172PAX	C	121.4	ng/µl	3.035	1.475	2.06	0.83
BR0173PAX	C	96.32	ng/µl	2.408	1.17	2.06	1.53

APPENDIX B

Table B. RIN values of the 150 RNA samples assessed by using Agilent 2100 Bioanalyzer. For the risk group: A-High Risk; B-Low Risk; C-MDD. N/A: RIN not detectable

Sample ID	Risk group	RIN
BR0001PAX	C	9.4
BR0002PAX	C	9.3
BR0005PAX	A	9.1
BR0006PAX	C	9.2
BR0009PAX	C	9.1
BR0013PAX	B	9.2
BR0015PAX	C	9
BR0018PAX	C	9.2
BR0019PAX	C	8.8
BR0020PAX	A	9.2
BR0021PAX	C	9.6
BR0022PAX	C	9.2
BR0024PAX	C	7.9
BR0027PAX	C	8.7
BR0028PAX	C	8.2
BR0029PAX	C	8.4
BR0030PAX	C	8.3
BR0032PAX	C	8.6
BR0033PAX	A	8.2
BR0035PAX	B	9.3
BR0036PAX	A	8
BR0037PAX	A	8
BR0038PAX	C	8.3

BR0039PAX	C	7.8
BR0040PAX	C	8.9
BR0043PAX	C	9.4
BR0044PAX	C	8
BR0045PAX	C	9.1
BR0046PAX	C	9.1
BR0047PAX	A	9.2
BR0048PAX	A	9.2
BR0049PAX	A	9.3
BR0050PAX	B	N/A
BR0051PAX	B	8.9
BR0052PAX	B	9
BR0053PAX	B	9.3
BR0054PAX	B	8.9
BR0055PAX	C	9.3
BR0056PAX	C	9.1
BR0057PAX	C	9.3
BR0058PAX	C	9.2
BR0059PAX	B	9.3
BR0060PAX	A	9.5
BR0061PAX	C	9.1
BR0064PAX	C	9.1
BR0065PAX	C	8.8
BR0066PAX	A	9
BR0067PAX	B	7.2
BR0069PAX	A	9.1
BR0070PAX	A	9

BR0071PAX	A	9.1
BR0072PAX	A	9
BR0073PAX	C	9.2
BR0074PAX	A	N/A
BR0075PAX	A	8.8
BR0076PAX	B	9.1
BR0077PAX	A	9
BR0078PAX	B	8.6
BR0079PAX	B	9.1
BR0080PAX	B	9
BR0081PAX	B	9.1
BR0082PAX	B	9.4
BR0083PAX	C	9
BR0084PAX	B	9
BR0085PAX	B	8.8
BR0086PAX	A	9.2
BR0087PAX	B	8.8
BR0088PAX	A	9.3
BR0089PAX	A	9.1
BR0091PAX	C	9.2
BR0092PAX	A	9.6
BR0094PAX	C	9.2
BR0095PAX	A	8.9
BR0096PAX	A	9.2
BR0097PAX	A	8.9
BR0099PAX	B	9.1
BR0100PAX	B	9

BR0101PAX	A	8.8
BR0103PAX	C	9.1
BR0104PAX	A	8.8
BR0105PAX	B	9.4
BR0106PAX	B	9
BR0107PAX	A	9.1
BR0108PAX	A	9.2
BR0109PAX	A	9.1
BR0110PAX	A	9
BR0111PAX	A	9
BR0112PAX	A	9
BR0113PAX	B	7.7
BR0114PAX	B	9
BR0115PAX	B	9.4
BR0117PAX	B	9.1
BR0120PAX	B	9.1
BR0121PAX	A	8.8
BR0122PAX	C	8.5
BR0128PAX	B	8.8
BR0131PAX	B	9.3
BR0137PAX	B	9.4
BR0140PAX	A	9.5
BR0142PAX	B	N/A
BR0143PAX	A	9.1
BR0144PAX	B	9.1
BR0151PAX	B	9.3
BR0152PAX	B	9

BR0157PAX	B	9.1
BR0090PAX	C	9.6
BR0093PAX	A	9.9
BR0098PAX	A	10
BR0102PAX	A	10
BR0116PAX	A	9.7
BR0118PAX	A	9.9
BR0119PAX	A	9.6
BR0123PAX	C	9.6
BR0124PAX	C	10
BR0125PAX	A	10
BR0127PAX	C	9.9
BR0129PAX	A	10
BR0130PAX	A	9.8
BR0132PAX	C	9.8
BR0133PAX	B	9.8
BR0134PAX	C	9.8
BR0135PAX	B	9.9
BR0136PAX	B	9.4
BR0138PAX	A	9.8
BR0139PAX	B	10
BR0141PAX	B	8.7
BR0145PAX	B	9.9
BR0146PAX	A	9.7
BR0147PAX	B	8.9
BR0148PAX	B	9.3
BR0149PAX	A	9.9

BR0150PAX	A	9.8
BR0153PAX	B	9.8
BR0154PAX	A	10
BR0155PAX	A	9.7
BR0156PAX	C	10
BR0158PAX	B	N/A
BR0160PAX	B	10
BR0161PAX	B	9.9
BR0162PAX	B	10
BR0163PAX	C	8.4
BR0164PAX	B	10
BR0165PAX	C	9.9
BR0167PAX	C	9.2
BR0168PAX	B	9.3
BR0169PAX	C	10
BR0170PAX	C	8.9
BR0171PAX	C	9.9
BR0172PAX	C	9.8
BR0173PAX	C	9.4

APPENDIX C

Table C. QC Report of the 150 samples.

Sample ID	Summary	Mean	Background	Signal-Noise ratio	BioBless Bio C	BioCless BioD	Lysless Phe	Phe less Dap
BR0001	*Include*	106.67	29.8	0.38	True	True	True	True
BR0002	*Include*	113.53	32.5	0.36	True	True	True	True
BR0005	*Include*	112.27	29.3	0.4	True	True	True	True
BR0006	*Include*	50.15	24.3	0.23	True	True	True	True
BR0009	*Include*	129.11	33	0.39	True	True	True	True
BR0013	*Include*	74.48	26.5	0.32	True	True	True	True
BR0015	*Include*	124.7	30.8	0.41	True	True	True	True
BR0019	*Include*	107.12	30.3	0.37	True	True	True	True
BR0020	*Include*	87.88	28	0.34	True	True	True	True
BR0021	*Include*	115.22	33.3	0.35	True	True	True	True
BR0022	*Include*	109.26	30.5	0.37	True	True	True	True
BR0024	*Include*	118.56	31.3	0.39	True	True	True	True
BR0027	*Include*	108.49	28.8	0.39	True	True	True	True
BR0028	*Include*	66.86	27.5	0.27	True	True	True	True
BR0029	*Include*	116.45	31.3	0.38	True	True	True	True
BR0030	*Include*	103.22	29.5	0.37	True	True	True	True
BR0032	*Include*	142.82	32.5	0.43	True	True	True	True
BR0033	*Include*	112.55	29.8	0.39	True	True	True	True
BR0035	*Include*	121.33	31	0.4	True	True	True	True
BR0036	*Include*	120.3	30.5	0.4	True	True	True	True
BR0037	*Include*	94.91	30	0.34	True	True	True	True
BR0038	*Include*	121.85	34	0.36	True	True	True	True

BR0039	*Include*	125.71	33.3	0.38	True	True	True	True
BR0040	*Include*	75.4	34.3	0.22	True	True	True	True
BR0043	*Include*	104.12	29.5	0.37	True	True	True	True
BR0044	*Include*	100.44	28.5	0.38	True	True	True	True
BR0045	*Include*	123.57	31.8	0.39	True	True	True	True
BR0046	*Include*	113.65	31.8	0.37	True	True	True	True
BR0047	*Include*	113.22	31.8	0.37	True	True	True	True
BR0048	*Include*	112.2	31.8	0.36	True	True	True	True
BR0049	*Include*	60.32	25.5	0.27	True	True	True	True
BR0050	*Include*	95.35	30.8	0.33	True	True	True	True
BR0051	*Include*	118.7	30	0.4	True	True	True	True
BR0052	*Include*	127.97	36.5	0.35	True	True	True	True
BR0053	*Include*	99.24	30.8	0.34	True	True	True	True
BR0054	*Include*	101.73	29.5	0.37	True	True	True	True
BR0055	*Include*	113.29	31.8	0.37	True	True	True	True
BR0056	*Include*	108.74	30.3	0.37	True	True	True	True
BR0057	*Include*	108.8	32	0.35	True	True	True	True
BR0058	*Include*	113.29	28.8	0.41	True	True	True	True
BR0059	*Include*	76.57	27.3	0.31	True	True	True	True
BR0059	*Include*	90.48	32.5	0.29	True	True	True	True
BR0060	*Include*	106.21	29.5	0.38	True	True	True	True
BR0064	*Include*	121.71	31	0.4	True	True	True	True
BR0065	*Include*	100.78	29.3	0.37	True	True	True	True
BR0066	*Include*	85.44	29.3	0.32	True	True	True	True
BR0067	*Include*	111.47	31	0.37	True	True	True	True
BR0069	*Include*	108.86	31	0.37	True	True	True	True
BR0070	*Include*	93.72	30	0.33	True	True	True	True
BR0071	*Include*	93.74	27.5	0.37	True	True	True	True

BR0072	*Include*	111.43	32	0.36	True	True	True	True
BR0073	*Include*	119.21	32	0.38	True	True	True	True
BR0074	*Include*	102.41	32.5	0.33	True	True	True	True
BR0075	*Include*	110.91	31.5	0.36	True	True	True	True
BR0076	*Include*	61.45	25	0.28	True	True	True	True
BR0077	*Include*	117.9	30.3	0.4	True	True	True	True
BR0078	*Include*	96.37	32	0.32	True	True	True	True
BR0079	*Include*	106.69	32.5	0.34	True	True	True	True
BR0080	*Include*	120.99	34	0.36	True	True	True	True
BR0081	*Include*	98.25	30.8	0.34	True	True	True	True
BR0082	*Include*	92.13	30.5	0.32	True	True	True	True
BR0083	*Include*	114.28	32.8	0.36	True	True	True	True
BR0084	*Include*	106	31.3	0.35	True	True	True	True
BR0085	*Include*	100.72	29.8	0.36	True	True	True	True
BR0086	*Include*	104.3	29.8	0.37	True	True	True	True
BR0086	*Include*	104.3	29.8	0.37	True	True	True	True
BR0087	*Include*	61.77	27.8	0.24	True	True	True	True
BR0089	*Include*	103.77	31.8	0.34	True	True	True	True
BR0090	*Include*	104.34	33.3	0.33	True	True	True	True
BR0091	*Include*	126.3	33.3	0.38	True	True	True	True
BR0092	*Include*	99.2	31.3	0.33	True	True	True	True
BR0093	*Include*	121.6	29.5	0.42	True	True	True	True
BR0094	*Include*	111.69	30.3	0.38	True	True	True	True
BR0095	*Include*	118.7	30.5	0.4	True	True	True	True
BR0096	*Include*	101.25	31.8	0.33	True	True	True	True
BR0097	*Include*	97.18	30.3	0.34	True	True	True	True
BR0098	*Include*	129.86	33	0.39	True	True	True	True
BR0099	*Include*	113.51	31.5	0.37	True	True	True	True

BR0100	*Include*	96.74	27.3	0.38	True	True	True	True
BR0101	*Include*	105.37	31.3	0.35	True	True	True	True
BR0102	*Include*	66.57	26.3	0.28	True	True	True	True
BR0103	*Include*	101.96	34.3	0.31	True	True	True	True
BR0104	*Include*	106.04	30.8	0.36	True	True	True	True
BR0105	*Include*	115.85	30.3	0.39	True	True	True	True
BR0106	*Include*	104.03	29.5	0.37	True	True	True	True
BR0107	*Include*	85.34	30	0.31	True	True	True	True
BR0108	*Include*	113.38	32	0.36	True	True	True	True
BR0109	*Include*	72.63	26.3	0.31	True	True	True	True
BR0110	*Include*	113.11	28.8	0.41	True	True	True	True
BR0111	*Include*	112.24	30.5	0.38	True	True	True	True
BR0112	*Include*	56.07	24.8	0.25	True	True	True	True
BR0113	*Include*	100.62	31.3	0.34	True	True	True	True
BR0113	*Include*	100.62	31.3	0.34	True	True	True	True
BR0114	*Include*	97.19	29.3	0.36	True	True	True	True
BR0115	*Include*	127.06	33	0.39	True	True	True	True
BR0116	*Include*	170.68	37.8	0.42	True	True	True	True
BR0117	*Include*	121.81	34.5	0.36	True	True	True	True
BR0118	*Include*	120.93	34	0.36	True	True	True	True
BR0119	*Include*	103.99	33	0.33	True	True	True	True
BR0120	*Include*	127.63	34.5	0.37	True	True	True	True
BR0121	*Include*	122.68	33.3	0.37	True	True	True	True
BR0122	*Include*	57.64	25	0.26	True	True	True	True
BR0123	*Include*	123.43	35.3	0.35	True	True	True	True
BR0124	*Include*	145.27	33	0.42	True	True	True	True
BR0125	*Include*	132.66	33.8	0.39	True	True	True	True
BR0127	*Include*	121.43	36.3	0.34	True	True	True	True

BR0128	*Include*	53.4	26.5	0.21	True	True	True	True
BR0129	*Include*	130.67	36.3	0.36	True	True	True	True
BR0130	*Include*	140.65	33.8	0.41	True	True	True	True
BR0131	*Include*	117	32.8	0.36	True	True	True	True
BR0132	*Include*	103.01	33.5	0.32	True	True	True	True
BR0133	*Include*	116.61	35.3	0.34	True	True	True	True
BR0134	*Include*	117.29	38.8	0.3	True	True	True	True
BR0135	*Include*	137.53	35.5	0.38	True	True	True	True
BR0136	*Include*	112.76	32.5	0.36	True	True	True	True
BR0137	*Include*	123.77	31.8	0.39	True	True	True	True
BR0138	*Include*	137.38	41.3	0.32	True	True	True	True
BR0139	*Include*	127.16	36	0.35	True	True	True	True
BR0141	*Include*	133.64	31.3	0.42	True	True	True	True
BR0142	*Include*	102.93	30.8	0.35	True	True	True	True
BR0143	*Include*	105.61	31.5	0.35	True	True	True	True
BR0144	*Include*	109.03	31	0.37	True	True	True	True
BR0145	*Include*	145.53	36	0.39	True	True	True	True
BR0146	*Include*	133.24	33.5	0.39	True	True	True	True
BR0147	*Include*	106.13	34.3	0.32	True	True	True	True
BR0148	*Include*	125.61	31.3	0.4	True	True	True	True
BR0149	*Include*	122.67	45.3	0.26	True	True	True	True
BR0150	*Include*	108.06	34.8	0.32	True	True	True	True
BR0151	*Include*	96.36	30.8	0.33	True	True	True	True
BR0152	*Include*	101.32	30.3	0.35	True	True	True	True
BR0153	*Include*	102.94	34.5	0.31	True	True	True	True
BR0154	*Include*	146.34	34.8	0.4	True	True	True	True
BR0155	*Include*	92.42	33.3	0.29	True	True	True	True
BR0156	*Include*	144.37	35	0.4	True	True	True	True

BR0157	*Include*	134.2	33.3	0.4	True	True	True	True
BR0158	*Include*	119.5	31.3	0.39	True	True	True	True
BR0160	*Include*	177.54	36.3	0.44	True	True	True	True
BR0161	*Include*	124.37	34.8	0.36	True	True	True	True
BR0162	*Include*	162.92	36.5	0.42	True	True	True	True
BR0163	*Include*	103.18	40	0.26	True	True	True	True
BR0164	*Include*	103.43	32.3	0.33	True	True	True	True
BR0165	*Include*	124.43	41	0.3	True	True	True	True
BR0167	*Include*	121.23	34.5	0.35	True	True	True	True
BR0168	*Include*	143.33	32.8	0.42	True	True	True	True
BR0169	*Include*	161.78	37	0.41	True	True	True	True
BR0170	*Include*	95.94	30.8	0.33	True	True	True	True
BR0171	*Include*	105.23	29	0.38	True	True	True	True
BR0172	*Include*	121.61	31.3	0.39	True	True	True	True
BR0172	*Include*	121.61	31.3	0.39	True	True	True	True
BR0173	*Include*	110.57	32.5	0.35	True	True	True	True

APPENDIX D

Table 1D. List of 79 genes differently modulated in MDD vs HR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TRAJ19	T Cell Receptor Alpha Joining 19 (Non-Functional)	0.007	1.44
TRAJ40	T Cell Receptor Alpha Joining 40	0.007	1.43
HM13-IT1	HM13 Intronic Transcript 1 (Non-Protein Coding)	0.019	1.37
OAS3	2'-5'-Oligoadenylate Synthetase 3, 100kda	0.019	1.35
COPE	Coatomer Protein Complex, Subunit Epsilon	0.004	1.35
NR1H2	Nuclear Receptor Subfamily 1, Group H, Member 2	0.022	1.34
SLED1	Proteoglycan 3 Pseudogene	0.005	1.31
CYBA	Cytochrome B-245, Alpha Polypeptide	0.005	1.31
HP	Haptoglobin	0.010	1.31
MX1	Myxovirus (Influenza Virus) Resistance 1, Interferon-Inducible P	0.012	1.31
XRRA1	X-Ray Radiation Resistance Associated 1	0.002	1.31
TRDJ4	T Cell Receptor Delta Joining 4	0.036	1.30
CMPK2	Cytidine Monophosphate (UMP-CMP) Kinase 2, Mitochondrial	0.033	1.30
C2orf68	Chromosome 2 Open Reading Frame 68	0.006	1.29
HIST1H2AM	Histone Cluster 1, H2am	0.010	1.28
CEACAM8	Carcinoembryonic Antigen-Related Cell Adhesion Molecule 8	0.050	1.28
ANKFY1	Ankyrin Repeat And FYVE Domain Containing 1	0.002	1.28
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.040	1.28
TNFRSF1B	Tumor Necrosis Factor Receptor Superfamily, Member 1B	0.011	1.27
FKBP8	FK506 Binding Protein 8, 38kda	0.019	1.27
P2RX1	Purinergic Receptor P2X, Ligand-Gated Ion Channel, 1	0.004	1.27
TUBB1	Tubulin, Beta 1 Class VI	0.045	1.26
CRISPLD2	Cysteine-Rich Secretory Protein LCCL Domain Containing 2	0.008	1.26
SNORD14E	Small Nucleolar RNA, C/D Box 14E	0.039	1.25

HMHA1	Histocompatibility (Minor) HA-1	0.010	1.25
FLNA	Filamin A, Alpha	0.015	1.25
PLEKHO2	Pleckstrin Homology Domain Containing, Family O Member 2	0.018	1.25
ECRP	Ribonuclease, Rnase A Family, 2	0.046	1.25
STAT2	Signal Transducer And Activator Of Transcription 2, 113kda	0.002	1.25
FBXW5	F-Box And WD Repeat Domain Containing 5	0.006	1.25
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.014	1.24
TRAJ6	T Cell Receptor Alpha Joining 6	0.039	1.24
FLOT2	Flotillin 2	0.008	1.24
IP6K1	Inositol Hexakisphosphate Kinase 1	0.009	1.24
AP5B1	Adaptor-Related Protein Complex 5, Beta 1 Subunit	0.001	1.24
FERMT3	Fermitin Family Member 3	0.011	1.24
UBN1	Ubinuclein 1	0.014	1.24
C6orf130	Chromosome 6 Open Reading Frame 130	0.005	1.24
KDM4B	Lysine (K)-Specific Demethylase 4B	0.007	1.24
CNP	2',3'-Cyclic Nucleotide 3' Phosphodiesterase	< 0.001	1.23
CYP4F3	Cytochrome P450, Family 4, Subfamily F, Polypeptide 3	0.021	1.23
ZER1	Zer-1 Homolog (C. Elegans)	0.023	1.23
ACSL1	Acyl-Coa Synthetase Long-Chain Family Member 1	0.009	1.23
CSK	C-Src Tyrosine Kinase	0.048	1.23
CCDC69	Coiled-Coil Domain Containing 69	0.044	1.22
CSF2RB	Colony Stimulating Factor 2 Receptor, Beta, Low-Affinity	0.012	1.22
IFIT2	Interferon-Induced Protein With Tetratricopeptide Repeats 2	0.019	1.22
ITGA2B	Integrin, Alpha 2b	0.007	1.22
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme, Catalytic Polypeptide-Li	0.003	1.22
PLEKHM2	Pleckstrin Homology Domain Containing, Family M	0.024	1.22
FAM106CP	Family With Sequence Similarity 106, Member C, Pseudogene	0.029	1.21
NACC1	Nucleus Accumbens Associated 1	0.025	1.21
C22orf32	Chromosome 22 Open Reading Frame 32	0.043	1.21
APOBR	Apolipoprotein B Receptor	0.016	1.21

SMOX	Spermine Oxidase	0.008	1.21
TMEM140	Transmembrane Protein 140	< 0.001	1.21
MVP	Major Vault Protein	0.020	1.21
OAS2	2'-5'-Oligoadenylate Synthetase 2, 69/71kda	0.036	1.21
CPT1A	Carnitine Palmitoyltransferase 1A (Liver)	0.014	1.21
PRIC285	Peroxisomal Proliferator-Activated Receptor A Interacting Co	0.003	1.21
MOB3A	MOB Kinase Activator 3A	0.046	1.21
MAP3K11	Mitogen-Activated Protein Kinase Kinase Kinase 11	0.013	1.21
JAK3	Janus Kinase 3	0.004	1.21
CHMP6	Charged Multivesicular Body Protein 6	0.004	1.21
NOTCH1	Notch 1	0.005	1.20
ITGAM	Integrin,Alpha M(Complement Component 3,Receptor 3 Subunit)	0.027	1.20
VPS72	Vacuolar Protein Sorting 72 Homolog (S. Cerevisiae)	0.037	1.20
FCAR	Fc Fragment Of Iga, Receptor	0.002	1.20
BST2	Bone Marrow Stromal Cell Antigen 2	0.013	1.20
UNC13D	Unc-13 Homolog D (C. Elegans)	0.020	1.20
C10orf54	Chromosome 10 Open Reading Frame 54	0.014	1.20
SNORD69	Small Nucleolar RNA, C/D Box 69	0.032	-1.21
TRAV26-2	T Cell Receptor Alpha Variable 26-2	0.048	-1.21
RN5S110	RNA, 5S Ribosomal 110	0.034	-1.25
SPINK8	Serine Peptidase Inhibitor, Kazal Type 8 (Putative)	0.009	-1.26
TCL1B	T-Cell Leukemia/Lymphoma 1B	0.006	-1.36
C4BPA	Complement Component 4 Binding Protein, Alpha	0.021	-1.43
RNU4ATAC	RNA, U4atac Small Nuclear (U12-Dependent Splicing)	0.010	-1.62
IGHV1-58	Immunoglobulin Heavy Variable 1-58	0.047	-2.00

Table 2D. List of 23 genes differently modulated in MDD vs LR in the microarray analysis (FC ±|1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
HP	Haptoglobin	0.004	1.35
CASP5	Caspase 5, Apoptosis-Related Cysteine Peptidase	0.015	1.34
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.017	1.33
SLED1	Proteoglycan 3 Pseudogene	0.010	1.28
XRRA1	X-Ray Radiation Resistance Associated 1	0.003	1.28
CMPK2	Cytidine Monophosphate (UMP-CMP) Kinase 2, Mitochondrial	0.049	1.27
CARD17	Caspase Recruitment Domain Family, Member 17	0.027	1.24
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N	0.011	1.24
MX1	Myxovirus (Influenza Virus) Resistance 1, Interferon-Inducible	0.042	1.24
IGLV3-1	Immunoglobulin Lambda Variable 3-1	0.023	1.24
MTVR2	Mouse Mammary Tumor Virus Receptor Homolog 2	0.003	1.23
TRAV2	T Cell Receptor Alpha Variable 2	0.017	1.23
IFIT2	Interferon-Induced Protein With Tetratricopeptide Repeats 2	0.018	1.23
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.033	1.21
HCG8	HLA Complex Group 8	0.017	-1.20
SPINK8	Serine Peptidase Inhibitor, Kazal Type 8 (Putative)	0.030	-1.21
SNORA11	Small Nucleolar RNA, H/ACA Box 11	0.029	-1.23
CBR3	Carbonyl Reductase 3	0.003	-1.25
ZNRD1	Zinc Ribbon Domain Containing 1	0.015	-1.26
TRAV26-2	T Cell Receptor Alpha Variable 26-2	0.017	-1.26
AHSP	Alpha Hemoglobin Stabilizing Protein	0.047	-1.27
LHB	Luteinizing Hormone Beta Polypeptide	0.038	-1.28
C4BPA	Complement Component 4 Binding Protein, Alpha	0.008	-1.52

Table 3D. List of 11 genes differently modulated in HR vs LR (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TCL1B	T-Cell Leukemia/Lymphoma 1B	0.004	1.39
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N	0.018	1.28
BSG	Basigin (Ok Blood Group)	0.013	-1.20
FAR1-IT1	FAR1 Intronic Transcript 1 (Non-Protein Coding)	0.019	-1.21
SMOX	Spermine Oxidase	0.009	-1.21
EPB49	Erythrocyte Membrane Protein Band 4.9 (Dematin)	0.028	-1.21
ANO7L1	Anoctamin 7-Like 1	0.024	-1.22
EPB42	Erythrocyte Membrane Protein Band 4.2	0.036	-1.23
OR2W3	Olfactory Receptor, Family 2, Subfamily W, Member 3	0.007	-1.26
PDIA3P	Protein Disulfide Isomerase Family A, Member 3 Pseudogene	0.015	-1.32
CCDC144A	Coiled-Coil Domain Containing 144A	0.004	-1.37

Table 4D. List of 592 genes differently modulated in males MDD vs males HR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
HM13-IT1	HM13 Intronic Transcript 1 (Non-Protein Coding)	0.010	1.61
COPE	Coatomer Protein Complex, Subunit Epsilon	0.001	1.61
NR1H2	Nuclear Receptor Subfamily 1, Group H, Member 2	0.008	1.61
IGHA1	Immunoglobulin Heavy Constant Alpha 1	0.026	1.59
IGHA2	Immunoglobulin Heavy Constant Alpha 2 (A2m Marker)	0.036	1.59
P2RX1	Purinergic Receptor P2X, Ligand-Gated Ion Channel, 1	< 0.001	1.55
PPP4C	Protein Phosphatase 4, Catalytic Subunit	0.031	1.53
TSPAN3	Tetraspanin 3	0.016	1.52
C2orf68	Chromosome 2 Open Reading Frame 68	0.001	1.52
HIST1H2AM	Histone Cluster 1, H2am	0.003	1.52
IP6K1	Inositol Hexakisphosphate Kinase 1	< 0.001	1.51
FLNA	Filamin A, Alpha	0.001	1.50
IGHG4	Immunoglobulin Heavy Constant Gamma 4 (G4m Marker)	0.026	1.49
CYBA	Cytochrome B-245, Alpha Polypeptide	0.004	1.47
SLC35E2B	Solute Carrier Family 35, Member E2B	0.006	1.46
CSNK1G2	Casein Kinase 1, Gamma 2	0.038	1.46
HMHA1	Histocompatibility (Minor) HA-1	0.002	1.46
MYH9	Myosin, Heavy Chain 9, Non-Muscle	0.008	1.45
HIST1H2BM	Histone Cluster 1, H2bm	0.010	1.45
HIST1H3I	Histone Cluster 1, H3i	0.017	1.45
FBXW5	F-Box And WD Repeat Domain Containing 5	0.001	1.44
TNFRSF1B	Tumor Necrosis Factor Receptor Superfamily, Member 1B	0.005	1.44
PPP1R15B	Protein Phosphatase 1, Regulatory Subunit 15B	0.031	1.43
TUBB1	Tubulin, Beta 1 Class VI	0.025	1.43

CCDC69	Coiled-Coil Domain Containing 69	0.013	1.42
ATP6AP1	Atpase, H+ Transporting, Lysosomal Accessory Protein 1	0.020	1.41
FERMT3	Fermitin Family Member 3	0.004	1.41
ST3GAL2	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2	< 0.001	1.41
CHMP6	Charged Multivesicular Body Protein 6	< 0.001	1.41
FLOT2	Flotillin 2	0.004	1.40
FCHO1	FCH Domain Only 1	0.008	1.40
MAP3K11	Mitogen-Activated Protein Kinase Kinase Kinase 11	0.001	1.40
PPIP5K1	Diphosphoinositol Pentakisphosphate Kinase 1	0.026	1.40
PLEKHO2	Pleckstrin Homology Domain Containing, Family O Member 2	0.012	1.39
POLR2E	Polymerase (RNA) II (DNA Directed) Polypeptide E, 25kda	0.026	1.39
GRAP	GRB2-Related Adaptor Protein	0.011	1.39
HGS	Hepatocyte Growth Factor-Regulated Tyrosine Kinase Substrate	0.010	1.39
RASA4	RAS P21 Protein Activator 4	0.025	1.39
ITGAM	Integrin, Alpha M (Complement Component 3 Receptor 3 Subunit)	0.005	1.39
PLEKHM2	Pleckstrin Homology Domain Containing, Family M	0.008	1.39
CRISPLD2	Cysteine-Rich Secretory Protein LCCL Domain Containing 2	0.006	1.39
PTBP1	Polypyrimidine Tract Binding Protein 1	0.035	1.39
LRRC59	Leucine Rich Repeat Containing 59	0.021	1.38
MVP	Major Vault Protein	0.005	1.38
MOB3A	MOB Kinase Activator 3A	0.015	1.38
CNP	2',3'-Cyclic Nucleotide 3' Phosphodiesterase	< 0.001	1.37
G6PC3	Glucose 6 Phosphatase, Catalytic, 3	< 0.001	1.37
FAR1-IT1	FAR1 Intronic Transcript 1 (Non-Protein Coding)	0.005	1.37
UNC13D	Unc-13 Homolog D (C. Elegans)	0.004	1.37
ATF6B	Activating Transcription Factor 6 Beta	0.009	1.37
BAK1	BCL2-Antagonist/Killer 1	< 0.001	1.37
PLAC4	Placenta-Specific 4	0.002	1.37

JAK3	Janus Kinase 3	0.001	1.37
LILRA6	Leukocyte Immunoglobulin-Like Receptor, Subfamily A	0.006	1.37
SHKBP1	SH3KBP1 Binding Protein 1	0.004	1.36
IGHG1	Immunoglobulin Heavy Constant Gamma 1 (G1m Marker)	0.014	1.36
VPS72	Vacuolar Protein Sorting 72 Homolog (S. Cerevisiae)	0.013	1.36
TET3	Tet Methylcytosine Dioxygenase 3	< 0.001	1.36
ZER1	Zer-1 Homolog (C. Elegans)	0.018	1.36
AP5B1	Adaptor-Related Protein Complex 5, Beta 1 Subunit	0.001	1.35
UBN1	Ubinuclein 1	0.012	1.35
FKBP8	FK506 Binding Protein 8, 38kda	0.036	1.35
ARHGAP30	Rho Gtpase Activating Protein 30	0.011	1.35
TRAJ33	T Cell Receptor Alpha Joining 33	0.005	1.35
CXXC1	CXXC Finger Protein 1	0.035	1.35
ALG12	Asparagine-Linked Glycosylation 12, Alpha-1,6-Mannosyltransferase	0.025	1.35
ANKFY1	Ankyrin Repeat And FYVE Domain Containing 1	0.006	1.35
ACAP1	Arfgap With Coiled-Coil, Ankyrin Repeat And PH Domains 1	0.014	1.35
PRKCSH	Protein Kinase C Substrate 80K-H	0.008	1.35
CDK9	Cyclin-Dependent Kinase 9	0.007	1.35
SGTA	Small Glutamine-Rich Tetratricopeptide Repeat (TPR)-Containing	0.006	1.35
GANAB	Glucosidase, Alpha; Neutral AB	0.002	1.35
PKM	Pyruvate Kinase, Muscle	0.026	1.34
APOBR	Apolipoprotein B Receptor	0.008	1.34
PREX1	Phosphatidylinositol-3,4,5-Trisphosphate-Dependent Rac Exchange	0.006	1.34
Septin 1	Septin 1	0.040	1.34
DYSF	Dysferlin, Limb Girdle Muscular Dystrophy 2B (Autosomal Recessi	0.011	1.34
C10orf54	Chromosome 10 Open Reading Frame 54	0.005	1.34
UPF1	UPF1 Regulator Of Nonsense Transcripts Homolog (Yeast)	0.009	1.34

TRBV20-1	T Cell Receptor Beta Variable 20-1	0.007	1.34
PIK3CD	Phosphoinositide-3-Kinase, Catalytic, Delta Polypeptide	0.016	1.34
CSF2RB	Colony Stimulating Factor 2 Receptor, Beta, Low-Affinity (Granul	0.011	1.33
FCAR	Fc Fragment Of Iga, Receptor For	0.001	1.33
SDF4	Stromal Cell Derived Factor 4	0.023	1.33
VPS18	Vacuolar Protein Sorting 18 Homolog (S. Cerevisiae)	0.005	1.33
TMEM161A	Transmembrane Protein 161A	0.002	1.33
MAN2C1	Mannosidase, Alpha, Class 2C, Member 1	0.008	1.33
PLIN3	Perilipin 3	0.039	1.33
UBA1	Ubiquitin-Like Modifier Activating Enzyme 1	0.012	1.33
VAV1	Vav 1 Guanine Nucleotide Exchange Factor	0.049	1.33
CD22	CD22 Molecule	0.032	1.33
TNFAIP2	Tumor Necrosis Factor, Alpha-Induced Protein 2	0.011	1.33
ENO1	Enolase 1, (Alpha)	0.007	1.33
ZNF836	Zinc Finger Protein 836	0.004	1.33
SNORD10	Small Nucleolar RNA, C/D Box 10	0.006	1.33
SUN2	Sad1 And UNC84 Domain Containing 2	0.004	1.33
CD93	CD93 Molecule	0.018	1.33
TLN1	Talin 1	0.005	1.33
NOTCH1	Notch 1	0.002	1.33
NACC1	Nucleus Accumbens Associated 1, BEN And BTB (POZ)	0.018	1.33
MLL4	Myeloid/Lymphoid Or Mixed-Lineage Leukemia 4	0.001	1.32
ATHL1	ATH1, Acid Trehalase-Like 1 (Yeast)	0.001	1.32
AP1M1	Adaptor-Related Protein Complex 1, Mu 1 Subunit	0.002	1.32
MAN2B1	Mannosidase, Alpha, Class 2B, Member 1	0.002	1.32
LTB	Lymphotoxin Beta (TNF Superfamily, Member 3)	0.010	1.32
PSD4	Pleckstrin And Sec7 Domain Containing 4	0.022	1.32
KDM4B	Lysine (K)-Specific Demethylase 4B	0.011	1.32

ASNA1	Arsa Arsenite Transporter, ATP-Binding, Homolog 1 (Bacterial)	0.001	1.32
SLC39A3	Solute Carrier Family 39 (Zinc Transporter), Member 3	0.008	1.32
NPRL3	Nitrogen Permease Regulator-Like 3 (S. Cerevisiae)	0.032	1.32
OR52K2	Olfactory Receptor, Family 52, Subfamily K, Member 2	0.019	1.32
SIN3B	SIN3 Transcription Regulator Homolog B (Yeast)	0.003	1.31
ADRBK1	Adrenergic, Beta, Receptor Kinase 1	< 0.001	1.31
OGDH	Oxoglutarate (Alpha-Ketoglutarate) Dehydrogenase (Lipoamide)	0.015	1.31
NLRC3	NLR Family, CARD Domain Containing 3	0.005	1.31
TBC1D10C	TBC1 Domain Family, Member 10C	0.003	1.31
IL4R	Interleukin 4 Receptor	0.005	1.31
CLCN7	Chloride Channel, Voltage-Sensitive 7	0.004	1.31
RHOA-IT1	RHOA Intronic Transcript 1 (Non-Protein Coding)	0.015	1.31
XRRA1	X-Ray Radiation Resistance Associated 1	0.022	1.31
EEF2	Eukaryotic Translation Elongation Factor 2	0.006	1.31
AGAP2	Arfgap With Gtpase Domain, Ankyrin Repeat And PH Domain 2	0.001	1.31
PCNXL3	Pecanex-Like 3 (Drosophila)	< 0.001	1.31
MZB1	Marginal Zone B And B1 Cell-Specific Protein	0.021	1.31
ACSL1	Acyl-Coa Synthetase Long-Chain Family Member 1	0.016	1.31
NBEAL2	Neurobeachin-Like 2	< 0.001	1.31
ABT1	Activator Of Basal Transcription 1	0.001	1.31
IGLV3-25	Immunoglobulin Lambda Variable 3-25	0.024	1.31
STRN4	Striatin, Calmodulin Binding Protein 4	< 0.001	1.30
FMNL1	Formin-Like 1	0.010	1.30
CPT1A	Carnitine Palmitoyltransferase 1A (Liver)	0.015	1.30
ITGA2B	Integrin, Alpha 2b (Platelet Glycoprotein Iib Of Iib/Iiia Complement	0.011	1.30
TSC22D4	TSC22 Domain Family, Member 4	0.006	1.30
TRANK1	Tetratricopeptide Repeat And Ankyrin Repeat Containing 1	0.003	1.30
TMEM181	Transmembrane Protein 181	< 0.001	1.30

MAPK8IP3	Mitogen-Activated Protein Kinase 8 Interacting Protein 3	< 0.001	1.30
FURIN	Furin (Paired Basic Amino Acid Cleaving Enzyme)	0.001	1.30
ASB6	Ankyrin Repeat And SOCS Box Containing 6	0.004	1.30
SH2D3C	SH2 Domain Containing 3C	0.034	1.30
DHRSX-IT1	DHRSX Intronic Transcript 1 (Non-Protein Coding)	0.006	1.30
SEC24C	SEC24 Family, Member C (S. Cerevisiae)	< 0.001	1.30
WBP1L	WW Domain Binding Protein 1-Like	< 0.001	1.30
PCIF1	PDX1 C-Terminal Inhibiting Factor 1	0.040	1.30
ABTB1	Ankyrin Repeat And BTB (POZ) Domain Containing 1	0.028	1.30
TREML2	Triggering Receptor Expressed On Myeloid Cells-Like	0.008	1.30
ATP2A3	Atpase, Ca++ Transporting, Ubiquitous	0.027	1.30
PPP6R1	Protein Phosphatase 6, Regulatory Subunit 1	< 0.001	1.30
SMARCD2	SWI/SNF Related, Matrix Associated, Actin Dependent Regulatory	0.001	1.30
CLU	Clusterin	0.036	1.29
PADI4	Peptidyl Arginine Deiminase, Type IV	0.027	1.29
SLC7A5	Solute Carrier Family 7 (Amino Acid Transporter Light Chain	0.031	1.29
SUPT6H	Suppressor Of Ty 6 Homolog (S. Cerevisiae)	0.005	1.29
NCLN	Nicalin	< 0.001	1.29
CYP4F3	Cytochrome P450, Family 4, Subfamily F, Polypeptide 3	0.034	1.29
USP5	Ubiquitin Specific Peptidase 5 (Isopeptidase T)	0.042	1.29
PLCG2	Phospholipase C, Gamma 2 (Phosphatidylinositol-Specific)	0.006	1.29
ELF4	E74-Like Factor 4 (Ets Domain Transcription Factor)	0.005	1.29
MMP25	Matrix Metallopeptidase 25	0.024	1.29
FGD2	FYVE, Rhogef And PH Domain Containing 2	0.003	1.29
TWF2	Twinfilin, Actin-Binding Protein, Homolog 2 (Drosophila)	0.040	1.29
BICD2	Bicaudal D Homolog 2 (Drosophila)	0.002	1.29
SRPR	Signal Recognition Particle Receptor (Docking Protein)	0.001	1.29
OS9	Osteosarcoma Amplified 9, Endoplasmic Reticulum Lectin	0.028	1.29
ZNF646	Zinc Finger Protein 646	0.002	1.29

MAP3K3	Mitogen-Activated Protein Kinase Kinase Kinase 3	0.002	1.29
IMPDH1	IMP (Inosine 5'-Monophosphate) Dehydrogenase 1	0.018	1.28
MKNK2	MAP Kinase Interacting Serine/Threonine Kinase 2	0.002	1.28
MYO1F	Myosin IF	0.019	1.28
ACLY	ATP Citrate Lyase	0.016	1.28
SNORA59A	Small Nucleolar RNA, H/ACA Box 59A	0.002	1.28
C6orf130	Chromosome 6 Open Reading Frame 130	0.021	1.28
PACSIN2	Protein Kinase C And Casein Kinase Substrate In Neurons 2	0.007	1.28
DOK3	Docking Protein 3	0.002	1.28
ZSCAN29	Zinc Finger And SCAN Domain Containing 29	0.001	1.28
KIF21B	Kinesin Family Member 21B	0.002	1.28
ADCY7	Adenylate Cyclase 7	0.011	1.28
CTSD	Cathepsin D	0.017	1.28
DNASE1	Deoxyribonuclease I	0.012	1.28
TECPR2	Tectonin Beta-Propeller Repeat Containing 2	0.003	1.28
GATAD2A	GATA Zinc Finger Domain Containing 2A	0.029	1.28
PDIA4	Protein Disulfide Isomerase Family A, Member 4	< 0.001	1.28
KIAA0100	Kiaa0100	0.005	1.28
PFKFB3	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3	0.036	1.28
PIM1	Pim-1 Oncogene	0.027	1.28
PRIC285	Peroxisomal Proliferator-Activated Receptor A	0.005	1.28
HCFC1	Host Cell Factor C1 (VP16-Accessory Protein)	0.006	1.28
C6orf48	Chromosome 6 Open Reading Frame 48	0.005	1.28
STK40	Serine/Threonine Kinase 40	0.010	1.28
ZDHHC18	Zinc Finger, DHHC-Type Containing 18	0.004	1.28
TSPAN14	Tetraspanin 14	0.043	1.28
EMR2	Egf-Like Module Containing, Mucin-Like, Hormone Receptor-Like 2	0.023	1.28
AP2A1	Adaptor-Related Protein Complex 2, Alpha 1 Subunit	0.006	1.27
IGKC	Immunoglobulin Kappa Constant	0.044	1.27

TMEM120A	Transmembrane Protein 120A	0.033	1.27
RAP1GAP2	RAP1 Gtpase Activating Protein 2	0.013	1.27
POLA2	Polymerase (DNA Directed), Alpha 2, Accessory Subunit	< 0.001	1.27
U2AF2	U2 Small Nuclear RNA Auxiliary Factor 2	0.011	1.27
FCRLA	Fc Receptor-Like A	0.023	1.27
TMEM43	Transmembrane Protein 43	0.031	1.27
KCTD5	Potassium Channel Tetramerisation Domain Containing 5	0.028	1.27
PKN1	Protein Kinase N1	0.005	1.27
FBXW2	F-Box And WD Repeat Domain Containing 2	< 0.001	1.27
NOMO1	NODAL Modulator 1	0.035	1.27
FCGRT	Fc Fragment Of Igg, Receptor, Transporter, Alpha	0.012	1.27
ZGPAT	Zinc Finger, CCCH-Type With G Patch Domain	0.013	1.27
C19orf38	Chromosome 19 Open Reading Frame 38	0.004	1.27
MAU2	MAU2 Chromatid Cohesion Factor Homolog (C. Elegans)	0.011	1.27
SNORA9	Small Nucleolar RNA, H/ACA Box 9	0.022	1.27
STK11	Serine/Threonine Kinase 11	0.009	1.27
PLEKHM1	Pleckstrin Homology Domain Containing, Family M (With RUN Domai	0.020	1.27
TKT	Transketolase	< 0.001	1.27
ARHGEF18	Rho/Rac Guanine Nucleotide Exchange Factor (GEF) 18	0.017	1.27
STAT2	Signal Transducer And Activator Of Transcription 2, 113kda	0.018	1.27
FXR2	Fragile X Mental Retardation, Autosomal Homolog 2	0.005	1.27
VPS39	Vacuolar Protein Sorting 39 Homolog (S. Cerevisiae	0.022	1.27
ACSS1	Acyl-Coa Synthetase Short-Chain Family Member 1	0.003	1.27
INPP5D	Inositol Polyphosphate-5-Phosphatase, 145kda	0.016	1.27
RAB1B	RAB1B, Member RAS Oncogene Family	< 0.001	1.27
DCAF11	DDB1 And CUL4 Associated Factor 11	0.010	1.26
PCSK7	Proprotein Convertase Subtilisin/Kexin Type 7	0.004	1.26
DCAF5	DDB1 And CUL4 Associated Factor 5	< 0.001	1.26

ATP13A2	Atpase Type 13A2	0.006	1.26
GRAMD1A	GRAM Domain Containing 1A	0.034	1.26
TBC1D2	TBC1 Domain Family, Member 2	0.021	1.26
PCYT2	Phosphate Cytidylyltransferase 2, Ethanolamine	0.046	1.26
BCL6	B-Cell CLL/Lymphoma 6	0.033	1.26
NUP210	Nucleoporin 210kda	0.005	1.26
RFWD3	Ring Finger And WD Repeat Domain 3	< 0.001	1.26
MON1B	MON1 Homolog B (Yeast)	0.007	1.26
FEM1A	Fem-1 Homolog A (C. Elegans)	0.033	1.26
TBC1D3H	TBC1 Domain Family, Member 3H	0.011	1.26
HDAC1	Histone Deacetylase 1	0.006	1.26
MSN	Moesin	0.013	1.26
PLB1	Phospholipase B1	0.031	1.26
C20orf3	Chromosome 20 Open Reading Frame 3	0.013	1.26
ITPR3	Inositol 1,4,5-Trisphosphate Receptor, Type 3	0.001	1.26
LMAN2	Lectin, Mannose-Binding 2	0.006	1.26
PGPEP1	Pyroglutamyl-Peptidase I	0.011	1.26
RAPGEF1	Rap Guanine Nucleotide Exchange Factor (GEF) 1	0.014	1.26
PRPF8	PRP8 Pre-Mrna Processing Factor 8 Homolog (S. Cerevisiae)	0.013	1.26
CXCR1	Chemokine (C-X-C Motif) Receptor 1	0.005	1.26
LRRC4	Leucine Rich Repeat Containing 4	0.002	1.26
MLKL	Mixed Lineage Kinase Domain-Like	0.037	1.26
PXN	Paxillin	0.024	1.26
SBNO2	Strawberry Notch Homolog 2 (Drosophila)	0.003	1.26
MKRN2	Makorin Ring Finger Protein 2	< 0.001	1.26
RABL2B	RAB, Member Of RAS Oncogene Family-Like 2B	0.028	1.26
ARPC1B	Actin Related Protein 2/3 Complex, Subunit 1B, 41kda	0.020	1.26
VCP	Valosin Containing Protein	0.001	1.26
GMIP	GEM Interacting Protein	0.022	1.26
VASP	Vasodilator-Stimulated Phosphoprotein	0.008	1.26

NUDT19	Nudix (Nucleoside Diphosphate Linked Moiety X)-Type Motif 19	0.004	1.26
STAT6	Signal Transducer And Activator Of Transcription 6	0.002	1.25
WDR1	WD Repeat Domain 1	0.001	1.25
RASGRP4	RAS Guanyl Releasing Protein 4	0.007	1.25
CHPF2	Chondroitin Polymerizing Factor 2	0.006	1.25
HIATL1	Hippocampus Abundant Transcript-Like 1	0.013	1.25
MAN2A2	Mannosidase, Alpha, Class 2A, Member 2	0.002	1.25
RASA3	RAS P21 Protein Activator 3	0.034	1.25
CARD11	Caspase Recruitment Domain Family, Member 11	0.031	1.25
DOK1	Docking Protein 1, 62kda (Downstream Of Tyrosine Kinase 1)	0.016	1.25
EHD3	EH-Domain Containing 3	0.037	1.25
CORO1A	Coronin, Actin Binding Protein, 1A	0.045	1.25
ATP11A	Atpase, Class VI, Type 11A	0.001	1.25
ING4	Inhibitor Of Growth Family, Member 4	0.021	1.25
RASSF3	Ras Association (Ralgds/AF-6) Domain Family Member 3	0.018	1.25
DNM2	Dynamin 2	0.003	1.25
SIPA1	Signal-Induced Proliferation-Associated 1	0.020	1.25
LRP1	Low Density Lipoprotein Receptor-Related Protein 1	0.001	1.25
MAP4K2	Mitogen-Activated Protein Kinase Kinase Kinase Kinase 2	0.027	1.25
CD7	CD7 Molecule	0.048	1.25
GPS1	G Protein Pathway Suppressor 1	0.010	1.25
SNRPA	Small Nuclear Ribonucleoprotein Polypeptide A	0.049	1.25
APOL2	Apolipoprotein L, 2	0.010	1.25
ITGAL	Integrin, Alpha L (Antigen CD11A (P180), Lymphocyte Function-Asso	0.002	1.25
P2RY8	Purinergic Receptor P2Y, G-Protein Coupled, 8	0.033	1.25
LASP1	LIM And SH3 Protein 1	< 0.001	1.25
TRPC4AP	Transient Receptor Potential Cation Channel, Subfamily C	0.002	1.25
WASH5P	WAS Protein Family Homolog 5 Pseudogene	0.028	1.25
DGAT1	Diacylglycerol O-Acyltransferase 1	0.008	1.25

EFTUD2	Elongation Factor Tu GTP Binding Domain Containing 2	0.012	1.25
TRAM2	Translocation Associated Membrane Protein 2	0.005	1.25
SNRNP200	Small Nuclear Ribonucleoprotein 200kda (U5)	0.001	1.25
PNPLA6	Patatin-Like Phospholipase Domain Containing 6	0.004	1.25
AKAP8L	A Kinase (PRKA) Anchor Protein 8-Like	0.004	1.25
NS3BP	Ns3bp	0.001	1.25
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme, Catalytic Polypeptide-Li	0.020	1.24
NFE2L1	Nuclear Factor (Erythroid-Derived 2)-Like 1	0.021	1.24
PIEZO1	Piezo-Type Mechanosensitive Ion Channel Component 1	< 0.001	1.24
TBC1D25	TBC1 Domain Family, Member 25	0.001	1.24
SMOX	Spermine Oxidase	0.032	1.24
UBL7	Ubiquitin-Like 7 (Bone Marrow Stromal Cell-Derived)	0.032	1.24
KIAA0195	Kiaa0195	0.009	1.24
LIMK1	LIM Domain Kinase 1	0.003	1.24
ZNF76	Zinc Finger Protein 76	0.001	1.24
RFNG	RFNG O-Fucosylpeptide 3-Beta-N-Acetylglucosaminyltransferase	0.011	1.24
TBC1D3G	TBC1 Domain Family, Member 3G	0.018	1.24
KIAA0226	Kiaa0226	0.012	1.24
EEPD1	Endonuclease/Exonuclease/Phosphatase Family Domain Containing 1	0.012	1.24
IARS2	Isoleucyl-Trna Synthetase 2, Mitochondrial	< 0.001	1.24
SH3BP5L	SH3-Binding Domain Protein 5-Like	0.001	1.24
LAT2	Linker For Activation Of T Cells Family, Member 2	0.044	1.24
ARHGAP1	Rho Gtpase Activating Protein 1	0.049	1.24
NINJ1	Ninjurin 1	0.038	1.24
CDK5RAP3	CDK5 Regulatory Subunit Associated Protein 3	0.026	1.24
IGF2R	Insulin-Like Growth Factor 2 Receptor	0.019	1.24
NOTCH2	Notch 2	0.001	1.24
GIT1	G Protein-Coupled Receptor Kinase Interacting Arfgap 1	0.004	1.24

ELMO2	Engulfment And Cell Motility 2	0.036	1.24
BIN3	Bridging Integrator 3	0.017	1.24
PTGER4	Prostaglandin E Receptor 4 (Subtype EP4)	0.003	1.24
NCKAP1L	NCK-Associated Protein 1-Like	< 0.001	1.24
CNPY3	Canopy 3 Homolog (Zebrafish)	0.035	1.24
POLR2J2	Polymerase (RNA) II (DNA Directed) Polypeptide J2	0.016	1.24
TAOK2	TAO Kinase 2	0.002	1.24
PTK2B	PTK2B Protein Tyrosine Kinase 2 Beta	0.021	1.24
ABCA7	ATP-Binding Cassette, Sub-Family A (ABC1), Member 7	0.010	1.24
TBC1D3F	TBC1 Domain Family, Member 3F	0.007	1.24
IL6R	Interleukin 6 Receptor	0.031	1.24
TRBV6-1	T Cell Receptor Beta Variable 6-1	0.019	1.24
RPUSD1	RNA Pseudouridylate Synthase Domain Containing 1	0.005	1.24
CALCOCO1	Calcium Binding And Coiled-Coil Domain 1	0.007	1.24
MLX	MAX-Like Protein X	0.002	1.24
DCTN1	Dynactin 1	0.005	1.24
VAC14	Vac14 Homolog (S. Cerevisiae)	0.040	1.24
TRIM8	Tripartite Motif Containing 8	0.012	1.24
TAS2R14	Taste Receptor, Type 2, Member 14	0.037	1.24
BTBD2	BTB (POZ) Domain Containing 2	0.010	1.24
SNORD23	Small Nucleolar RNA, C/D Box 23	0.023	1.24
ARAP1	Arfgap With Rhogap Domain, Ankyrin Repeat And PH Domain 1	0.001	1.24
USP19	Ubiquitin Specific Peptidase 19	0.003	1.24
CSF1R	Colony Stimulating Factor 1 Receptor	0.004	1.24
MYO9B	Myosin IXB	0.007	1.24
SLFN12L	Schlafen Family Member 12-Like	0.012	1.24
ESYT1	Extended Synaptotagmin-Like Protein 1	0.023	1.24
MAN2B2	Mannosidase, Alpha, Class 2B, Member 2	0.003	1.24
XAB2	XPA Binding Protein 2	0.020	1.24
PIM2	Pim-2 Oncogene	0.015	1.24

CFP	Complement Factor Properdin	0.004	1.24
DEF6	Differentially Expressed In FDCP 6 Homolog (Mouse)	0.002	1.24
PNPLA2	Patatin-Like Phospholipase Domain Containing 2	0.005	1.24
FAM91A2	Family With Sequence Similarity 91, Member A2	0.005	1.24
RGS19	Regulator Of G-Protein Signaling 19	0.004	1.23
BMP6	Bone Morphogenetic Protein 6	0.001	1.23
FES	Feline Sarcoma Oncogene	0.005	1.23
TRAJ4	T Cell Receptor Alpha Joining 4	0.032	1.23
GPAA1	Glycosylphosphatidylinositol Anchor Attachment Protein 1 Homolog	0.002	1.23
MINK1	Misshapen-Like Kinase 1	0.001	1.23
MAST3	Microtubule Associated Serine/Threonine Kinase 3	0.037	1.23
TGFB1	Transforming Growth Factor, Beta 1	0.005	1.23
TAF6	TAF6 RNA Polymerase II, TATA Box Binding Protein (TBP)-Associated	0.024	1.23
ACOT8	Acyl-Coa Thioesterase 8	0.002	1.23
ZNF319	Zinc Finger Protein 319	0.019	1.23
NCF2	Neutrophil Cytosolic Factor 2	0.026	1.23
HK3	Hexokinase 3 (White Cell)	0.038	1.23
TMC6	Transmembrane Channel-Like 6	0.017	1.23
WDTC1	WD And Tetratricopeptide Repeats 1	0.007	1.23
FAM215A	Family With Sequence Similarity 215, Member A	0.015	1.23
SRCAP	Snf2-Related CREBBP Activator Protein	0.011	1.23
TBC1D3B	TBC1 Domain Family, Member 3B	0.028	1.23
TMEM140	Transmembrane Protein 140	0.004	1.23
LRSAM1	Leucine Rich Repeat And Sterile Alpha Motif Containing 1	0.001	1.23
BSG	Basigin (Ok Blood Group)	0.047	1.23
STAB1	Stabilin 1	0.001	1.23
GPI	Glucose-6-Phosphate Isomerase	0.012	1.23
ITGAX	Integrin, Alpha X (Complement Component 3 Receptor 4 Subunit)	0.020	1.23

IGLV3-12	Immunoglobulin Lambda Variable 3-12	0.048	1.23
IGKV4-1	Immunoglobulin Kappa Variable 4-1	0.008	1.23
DIP2A	DIP2 Disco-Interacting Protein 2 Homolog A (Drosophila)	0.001	1.23
ATG4B	Autophagy Related 4B, Cysteine Peptidase	0.014	1.23
TMEM63A	Transmembrane Protein 63A	0.011	1.23
ADAP1	Arfgap With Dual PH Domains 1	0.006	1.23
CPSF1	Cleavage And Polyadenylation Specific Factor 1, 160kda	0.036	1.23
LRP10	Low Density Lipoprotein Receptor-Related Protein 10	0.017	1.23
GRK6	G Protein-Coupled Receptor Kinase 6	0.011	1.23
AKNA	AT-Hook Transcription Factor	0.003	1.23
GNB2	Guanine Nucleotide Binding Protein (G Protein), Beta Polypep	< 0.001	1.23
PNKP	Polynucleotide Kinase 3'-Phosphatase	0.010	1.23
BRPF1	Bromodomain And PHD Finger Containing, 1	0.009	1.23
CD99L2	CD99 Molecule-Like 2	0.019	1.23
ARHGEF2	Rho/Rac Guanine Nucleotide Exchange Factor (GEF) 2	0.006	1.23
CLCN4	Chloride Channel, Voltage-Sensitive 4	0.011	1.23
XYLT2	Xylosyltransferase II	0.025	1.23
TBL3	Transducin (Beta)-Like 3	0.015	1.23
ADAR	Adenosine Deaminase, RNA-Specific	0.016	1.23
C11orf68	Chromosome 11 Open Reading Frame 68	0.008	1.23
MYO1G	Myosin IG	0.001	1.23
QSOX1	Quiescin Q6 Sulfhydryl Oxidase 1	0.041	1.23
TRMT2B	Trna Methyltransferase 2 Homolog B (S. Cerevisiae)	0.006	1.23
NEURL4	Neuralized Homolog 4 (Drosophila)	< 0.001	1.23
AGTRAP	Angiotensin II Receptor-Associated Protein	0.032	1.23
STAT3	Signal Transducer And Activator Of Transcription 3 (Acute-Phase Response)	0.009	1.22
HCP5	HLA Complex P5 (Non-Protein Coding)	0.024	1.22
ICAM3	Intercellular Adhesion Molecule 3	0.012	1.22
FYCO1	FYVE And Coiled-Coil Domain Containing 1	0.003	1.22

CECR1	Cat Eye Syndrome Chromosome Region, Candidate 1	0.004	1.22
ZNF335	Zinc Finger Protein 335	0.003	1.22
PPP1R18	Protein Phosphatase 1, Regulatory Subunit 18	0.001	1.22
PMS2	PMS2 Postmeiotic Segregation Increased 2 (S. Cerevisiae)	0.028	1.22
DYNC1H1	Dynein, Cytoplasmic 1, Heavy Chain 1	0.004	1.22
TBC1D17	TBC1 Domain Family, Member 17	0.005	1.22
ACTR1B	ARP1 Actin-Related Protein 1 Homolog B, Centractin Beta	0.014	1.22
HSPA1B	Heat Shock 70kda Protein 1B	0.027	1.22
TYK2	Tyrosine Kinase 2	0.006	1.22
ZNF142	Zinc Finger Protein 142	0.014	1.22
MED16	Mediator Complex Subunit 16	0.021	1.22
ABCG1	ATP-Binding Cassette, Sub-Family G (WHITE), Member 1	0.026	1.22
TRIM44	Tripartite Motif Containing 44	0.022	1.22
ELAC2	Elac Homolog 2 (E. Coli)	0.003	1.22
CMTM8	CKLF-Like MARVEL Transmembrane Domain Containing 8	0.019	1.22
NPIP	Nuclear Pore Complex Interacting Protein	0.003	1.22
CDS2	CDP-Diacylglycerol Synthase (Phosphatidate Cytidylyltransferase) 2	< 0.001	1.22
APOL6	Apolipoprotein L, 6	0.020	1.22
INTS3	Integrator Complex Subunit 3	0.008	1.22
KCTD7	Potassium Channel Tetramerisation Domain Containing 7	0.012	1.22
ELOVL1	ELOVL Fatty Acid Elongase 1	0.003	1.22
HIST1H1E	Histone Cluster 1, H1e	0.003	1.22
TRAFD1	TRAF-Type Zinc Finger Domain Containing 1	0.008	1.22
ANXA5	Annexin A5	0.002	1.22
NOMO2	NODAL Modulator 2	0.031	1.22
HIST2H2BA	Histone Cluster 2, H2ba (Pseudogene)	0.007	1.22
PRKCD	Protein Kinase C, Delta	0.003	1.22
C17orf62	Chromosome 17 Open Reading Frame 62	0.001	1.22
CD82	CD82 Molecule	0.017	1.22

NTNG2	Netrin G2	0.013	1.22
ATP6V0D1	Atpase, H+ Transporting, Lysosomal 38kda, V0 Subunit D1	0.013	1.22
FLII	Flightless I Homolog (Drosophila)	0.006	1.22
MFNG	MFNG O-Fucosylpeptide 3-Beta-N Acetylglucosaminyltransferase	0.009	1.22
CLN3	Ceroid-Lipofuscinosis, Neuronal 3	0.049	1.22
GIGYF1	GRB10 Interacting GYF Protein 1	0.007	1.22
PLD3	Phospholipase D Family, Member 3	0.004	1.22
ARSG	Arylsulfatase G	< 0.001	1.22
GLG1	Golgi Glycoprotein 1	0.003	1.22
SPPL2B	Signal Peptide Peptidase Like 2B	0.006	1.22
DENND5B	DENN/MADD Domain Containing 5B	0.003	1.22
ARPC4	Actin Related Protein 2/3 Complex, Subunit 4, 20kda	0.001	1.22
ITGB2	Integrin, Beta 2 (Complement Component 3 Receptor 3 And 4 S	0.037	1.22
UBE2O	Ubiquitin-Conjugating Enzyme E2O	0.034	1.22
GAA	Glucosidase, Alpha; Acid	0.009	1.22
NCR1	Natural Cytotoxicity Triggering Receptor 1	0.010	1.22
KCNQ1	Potassium Voltage-Gated Channel, KQT-Like Subfamily, Member 1	0.002	1.22
GAK	Cyclin G Associated Kinase	0.012	1.22
ABHD2	Abhydrolase Domain Containing 2	0.003	1.22
SEMA4D	Sema Domain, Immunoglobulin Domain (Ig), Transmembrane Domain	0.011	1.21
NEDD9	Neural Precursor Cell Expressed, Developmentally Down-Regul	0.008	1.21
PREB	Prolactin Regulatory Element Binding	0.034	1.21
PRPF6	PRP6 Pre-Mrna Processing Factor 6 Homolog (S. Cerevisiae)	0.038	1.21
COX10-AS1	COX10 Antisense RNA 1 (Non-Protein Coding)	0.036	1.21
CIRBP	Cold Inducible RNA Binding Protein	0.037	1.21
ACOX1	Acyl-Coa Oxidase 1, Palmitoyl	0.012	1.21
CIITA	Class II, Major Histocompatibility Complex, Transactivator	0.017	1.21
AES	Amino-Terminal Enhancer Of Split	0.034	1.21

ABI3	ABI Family, Member 3	0.036	1.21
CSF3R	Colony Stimulating Factor 3 Receptor (Granulocyte)	0.027	1.21
ERLIN1	ER Lipid Raft Associated 1	< 0.001	1.21
STK10	Serine/Threonine Kinase 10	0.011	1.21
C19orf10	Chromosome 19 Open Reading Frame 10	0.029	1.21
CES2	Carboxylesterase 2	0.015	1.21
PRKD2	Protein Kinase D2	0.005	1.21
IKBKE	Inhibitor Of Kappa Light Polypeptide Gene Enhancer In B-Cells, Ki	0.022	1.21
CD101	CD101 Molecule	0.010	1.21
FAM134C	Family With Sequence Similarity 134, Member C	0.044	1.21
PTDSS1	Phosphatidylserine Synthase 1	0.001	1.21
SIRPB2	Signal-Regulatory Protein Beta 2	0.004	1.21
PRRC2B	Proline-Rich Coiled-Coil 2B	0.032	1.21
SNORD84	Small Nucleolar RNA, C/D Box 84	0.012	1.21
FGD3	FYVE, Rhogef And PH Domain Containing 3	0.012	1.21
HIF1AN	Hypoxia Inducible Factor 1, Alpha Subunit Inhibitor	0.015	1.21
SH3BP5	SH3-Domain Binding Protein 5 (BTK-Associated)	0.018	1.21
SNORD88A	Small Nucleolar RNA, C/D Box 88A	0.018	1.21
RHOT2	Ras Homolog Family Member T2	0.018	1.21
DGCR2	Digeorge Syndrome Critical Region Gene 2	0.046	1.21
PHF15	PHD Finger Protein 15	0.005	1.21
ERN1	Endoplasmic Reticulum To Nucleus Signaling 1	0.025	1.21
CAMKK2	Calcium/Calmodulin-Dependent Protein Kinase Kinase 2, Beta	0.028	1.21
MPDU1	Mannose-P-Dolichol Utilization Defect 1	0.001	1.21
ITGA5	Integrin, Alpha 5 (Fibronectin Receptor, Alpha Polypeptide)	0.028	1.21
EIF4H	Eukaryotic Translation Initiation Factor 4H	0.006	1.21
PSENEN	Presenilin Enhancer 2 Homolog (C. Elegans)	0.008	1.21
EPHB1	EPH Receptor B1	0.027	1.21
ZZEF1	Zinc Finger, ZZ-Type With EF-Hand Domain 1	0.003	1.21

HIST1H2BL	Histone Cluster 1, H2bl	0.034	1.21
HIST1H2BC	Histone Cluster 1, H2bc	0.042	1.21
MTA2	Metastasis Associated 1 Family, Member 2	0.028	1.21
APLP2	Amyloid Beta (A4) Precursor-Like Protein 2	0.018	1.21
DPP7	Dipeptidyl-Peptidase 7	0.030	1.21
SLC16A3	Solute Carrier Family 16, Member 3	0.018	1.21
MTMR14	Myotubularin Related Protein 14	0.041	1.21
ZNF552	Zinc Finger Protein 552	0.006	1.21
KLHDC3	Kelch Domain Containing 3	0.039	1.21
SLC12A9	Solute Carrier Family 12 (Potassium/Chloride Transporters)	0.021	1.21
MAVS	Mitochondrial Antiviral Signaling Protein	0.039	1.21
C14orf43	Chromosome 14 Open Reading Frame 43	0.007	1.21
STAT5B	Signal Transducer And Activator Of Transcription 5B	0.001	1.21
SASH3	SAM And SH3 Domain Containing 3	0.026	1.21
ARMC6	Armadillo Repeat Containing 6	0.004	1.21
FOSL2	FOS-Like Antigen 2	0.035	1.21
TFE3	Transcription Factor Binding To IGHM Enhancer 3	0.006	1.21
UBR4	Ubiquitin Protein Ligase E3 Component N-Recognin 4	0.001	1.21
DENND4B	DENN/MADD Domain Containing 4B	0.009	1.21
CORO2A	Coronin, Actin Binding Protein, 2A	0.032	1.21
RALY	RNA Binding Protein, Autoantigenic	0.008	1.21
KCNAB2	Potassium Voltage-Gated Channel, Shaker-Related Subfamily, Beta	0.046	1.21
API5	Apoptosis Inhibitor 5	0.002	1.21
C7orf43	Chromosome 7 Open Reading Frame 43	0.003	1.20
LAPTM5	Lysosomal Protein Transmembrane 5	0.036	1.20
DEDD	Death Effector Domain Containing	0.015	1.20
SYMPK	Symplekin	0.001	1.20
NLRC5	NLR Family, CARD Domain Containing 5	0.006	1.20
AMPD2	Adenosine Monophosphate Deaminase 2	0.001	1.20

PLEKHM1P	Pleckstrin Homology Domain Containing, Family M	0.026	1.20
GPR108	G Protein-Coupled Receptor 108	0.043	1.20
SZT2	Seizure Threshold 2 Homolog (Mouse)	0.001	1.20
MLC1	Megalencephalic Leukoencephalopathy With Subcortical Cysts 1	0.016	1.20
PSIMCT-1	Malignant T Cell Amplified Sequence 1 Pseudogene	0.010	1.20
ARHGAP4	Rho Gtpase Activating Protein 4	0.019	1.20
ZMAT5	Zinc Finger, Matrin-Type 5	0.021	1.20
CAPN1	Calpain 1, (Mu/I) Large Subunit	0.010	1.20
KLHL6	Kelch-Like 6 (Drosophila)	0.009	1.20
PIGO	Phosphatidylinositol Glycan Anchor Biosynthesis, Class O	0.004	1.20
HM13	Histocompatibility (Minor) 13	0.002	1.20
PTPRM	Protein Tyrosine Phosphatase, Receptor Type, M	0.020	1.20
UNK	Unkempt Homolog (Drosophila)	0.002	1.20
KDM3B	Lysine (K)-Specific Demethylase 3B	0.003	1.20
AP5Z1	Adaptor-Related Protein Complex 5, Zeta 1 Subunit	0.002	1.20
MLLT1	Myeloid/Lymphoid Or Mixed-Lineage Leukemia	0.008	1.20
MTRNR2L8	MT-RNR2-Like 8	0.048	1.20
SLC27A3	Solute Carrier Family 27 (Fatty Acid Transporter)	0.022	1.20
MOV10	Mov10, Moloney Leukemia Virus 10, Homolog (Mouse)	0.025	1.20
KDM5D	Lysine (K)-Specific Demethylase 5D	0.009	1.20
ELOF1	Elongation Factor 1 Homolog (S. Cerevisiae)	0.048	1.20
FKRP	Fukutin Related Protein	0.004	1.20
NEK6	NIMA (Never In Mitosis Gene A)-Related Kinase 6	0.017	1.20
SUMF2	Sulfatase Modifying Factor 2	0.037	1.20
COBRA1	Cofactor Of BRCA1	0.045	1.20
ZNF813	Zinc Finger Protein 813	0.020	-1.20
KRTAP6-3	Keratin Associated Protein 6-3	0.001	-1.20
C8orf40	Chromosome 8 Open Reading Frame 40	0.011	-1.20
RPS25	Ribosomal Protein S25	0.002	-1.20

ATP5H	ATP Synthase, H+ Transporting, Mitochondrial Fo Complex, Subunit	0.028	-1.20
F2RL2	Coagulation Factor II (Thrombin) Receptor-Like 2	0.001	-1.20
VCX3B	Variable Charge, X-Linked 3B	0.037	-1.21
TRAPPC5	Trafficking Protein Particle Complex 5	0.011	-1.21
WDR53	WD Repeat Domain 53	< 0.001	-1.21
TMEM14A	Transmembrane Protein 14A	0.041	-1.21
NDUFB1	NADH Dehydrogenase (Ubiquinone) 1 Beta Subcomplex, 1, 7kda	0.007	-1.21
HBZ	Hemoglobin, Zeta	0.042	-1.22
ASNSD1	Asparagine Synthetase Domain Containing 1	0.023	-1.22
PPP2R3B-AS1	PPP2R3B Antisense RNA 1 (Non-Protein Coding)	0.027	-1.22
NDUFA2	NADH Dehydrogenase (Ubiquinone) 1 Alpha Subcomplex, 2, 8kda	0.006	-1.23
TPRKB	TP53RK Binding Protein	0.015	-1.23
SNORD113-8	Small Nucleolar RNA, C/D Box 113-8	0.014	-1.23
FRA10AC1	Fragile Site, Folic Acid Type, Rare, Fra(10)(Q23.3) Or Fra(10)	0.005	-1.23
LINC00239	Long Intergenic Non-Protein Coding RNA 239	0.003	-1.23
UQCRHL	Ubiquinol-Cytochrome C Reductase Hinge Protein-Like	0.031	-1.23
HBD	Hemoglobin, Delta	0.024	-1.23
PIN1P1	Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 1 Pseudogen	0.016	-1.23
POLR2J	Polymerase (RNA) II (DNA Directed) Polypeptide J, 13.3kda	0.015	-1.24
RPL13AP20	Ribosomal Protein L13a Pseudogene 20	0.008	-1.24
TMOD4	Tropomodulin 4 (Muscle)	0.006	-1.24
BEX5	Brain Expressed, X-Linked 5	0.020	-1.24
GPR18	G Protein-Coupled Receptor 18	0.014	-1.24
CBR3	Carbonyl Reductase 3	0.038	-1.24
TXNDC17	Thioredoxin Domain Containing 17	0.034	-1.24

SNORA70G	Small Nucleolar RNA, H/ACA Box 70G	0.016	-1.25
HIGD1A	HIG1 Hypoxia Inducible Domain Family, Member 1A	0.008	-1.25
ROMO1	Reactive Oxygen Species Modulator 1	0.006	-1.25
KLHL22-IT1	KLHL22 Intronic Transcript 1 (Non-Protein Coding)	0.036	-1.25
NDUFB6	NADH Dehydrogenase (Ubiquinone) 1 Beta Subcomplex, 6, 17kd	0.009	-1.26
FAM209B	Family With Sequence Similarity 209, Member B	0.040	-1.26
CHCHD1	Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 1	0.019	-1.26
MT1F	Metallothionein 1F	0.047	-1.26
PAGE2B	P Antigen Family, Member 2B	0.021	-1.27
TSIX	TSIX Transcript, XIST Antisense RNA (Non-Protein Coding)	0.030	-1.27
CCDC30	Coiled-Coil Domain Containing 30	0.022	-1.27
RPF2	Ribosome Production Factor 2 Homolog (S. Cerevisiae)	0.030	-1.28
SNHG8	Small Nucleolar RNA Host Gene 8 (Non-Protein Coding)	0.030	-1.28
HCG14	HLA Complex Group 14	0.001	-1.28
GAGE12C	G Antigen 12C /	0.013	-1.29
COX6A1	Cytochrome C Oxidase Subunit Via Polypeptide 1	0.012	-1.29
SNORD69	Small Nucleolar RNA, C/D Box 69	0.037	-1.30
HLA-E	Major Histocompatibility Complex, Class I, E	0.019	-1.30
SNORD14A	Small Nucleolar RNA, C/D Box 14A	0.040	-1.32
ZNF404	Zinc Finger Protein 404	0.028	-1.32
HSD17B13	Hydroxysteroid (17-Beta) Dehydrogenase 13	0.001	-1.34
TMSB4XP4	Thymosin Beta 4, X-Linked Pseudogene 4	0.005	-1.35
SPINK8	Serine Peptidase Inhibitor, Kazal Type 8 (Putative)	0.014	-1.36
HBM	Hemoglobin, Mu	0.018	-1.37
TCL1B	T-Cell Leukemia/Lymphoma 1B	0.047	-1.37
PLGLB1	Plasminogen-Like B1	0.044	-1.40
ECH1	Enoyl Coa Hydratase 1, Peroxisomal	0.037	-1.46
AHSP	Alpha Hemoglobin Stabilizing Protein	0.013	-1.52

Table 5D. List of 130 genes differently modulated in males MDD vs males LR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
IGHA2	Immunoglobulin Heavy Constant Alpha 2 (A2m Marker)	0.018	1.69
IGHA1	Immunoglobulin Heavy Constant Alpha 1	0.017	1.64
GGTLC2	Gamma-Glutamyltransferase Light Chain 2	0.004	1.61
IGHG1	Immunoglobulin Heavy Constant Gamma 1 (G1m Marker)	0.001	1.56
IGLV2-11	Immunoglobulin Lambda Variable 2-11	0.006	1.54
HM13-IT1	HM13 Intronic Transcript 1 (Non-Protein Coding)	0.034	1.48
IGHG4	Immunoglobulin Heavy Constant Gamma 4 (G4m Marker)	0.031	1.47
HIST1H2BM	Histone Cluster 1, H2bm	0.008	1.46
LTF	Lactotransferrin	0.001	1.45
COPE	Coatomer Protein Complex, Subunit Epsilon	0.015	1.43
HP	Haptoglobin	0.019	1.42
XRRA1	X-Ray Radiation Resistance Associated 1	0.007	1.38
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N	0.008	1.37
CYBA	Cytochrome B-245, Alpha Polypeptide	0.019	1.37
FLNA	Filamin A, Alpha	0.015	1.36
ATHL1	ATH1, Acid Trehalase-Like 1 (Yeast)	< 0.001	1.35
RASA4	RAS P21 Protein Activator 4	0.040	1.35
IGLC2	Immunoglobulin Lambda Constant 2 (Kern-Oz- Marker)	0.005	1.35
MMP8	Matrix Metallopeptidase 8 (Neutrophil Collagenase)	0.025	1.34
MZB1	Marginal Zone B And B1 Cell-Specific Protein	0.012	1.34
HMHA1	Histocompatibility (Minor) HA-1	0.017	1.33
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.022	1.33
OLFM4	Olfactomedin 4	0.003	1.33
IGLV3-25	Immunoglobulin Lambda Variable 3-25	0.017	1.33
IGLV3-10	Immunoglobulin Lambda Variable 3-10	0.049	1.31

ITPR3	Inositol 1,4,5-Trisphosphate Receptor, Type 3	< 0.001	1.31
PLAC4	Placenta-Specific 4	0.008	1.31
IP6K1	Inositol Hexakisphosphate Kinase 1	0.019	1.31
ABT1	Activator Of Basal Transcription 1	0.001	1.30
LILRB1	Leukocyte Immunoglobulin-Like Receptor, Subfamily B	0.023	1.30
MVP	Major Vault Protein	0.029	1.29
IGLC7	Immunoglobulin Lambda Constant 7	0.032	1.29
RNPEPL1	Arginyl Aminopeptidase (Aminopeptidase B)-Like 1	0.001	1.28
NOTCH1	Notch 1	0.007	1.28
PLEKHM2	Pleckstrin Homology Domain Containing, Family M	0.042	1.28
NLRC3	NLR Family, CARD Domain Containing 3	0.011	1.28
BAK1	BCL2-Antagonist/Killer 1	0.001	1.28
MTVR2	Mouse Mammary Tumor Virus Receptor Homolog 2	0.016	1.28
ST3GAL2	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 2	0.010	1.27
TMEM161A	Transmembrane Protein 161A	0.011	1.27
MAN2A2	Mannosidase, Alpha, Class 2A, Member 2	0.001	1.27
TBC1D3B	TBC1 Domain Family, Member 3B	0.013	1.27
ANKFY1	Ankyrin Repeat And FYVE Domain Containing 1	0.032	1.26
MXRA7	Matrix-Remodelling Associated 7	0.018	1.26
RHOA-IT1	RHOA Intronic Transcript 1 (Non-Protein Coding)	0.038	1.26
CHMP6	Charged Multivesicular Body Protein 6	0.010	1.26
IGKV1D-39	Immunoglobulin Kappa Variable 1D-39	0.035	1.26
TBC1D3G	TBC1 Domain Family, Member 3G	0.014	1.25
UNC13D	Unc-13 Homolog D (C. Elegans)	0.038	1.25
PRKCSH	Protein Kinase C Substrate 80K-H	0.047	1.25
SUN2	Sad1 And UNC84 Domain Containing 2	0.023	1.25
TBC1D10C	TBC1 Domain Family, Member 10C	0.015	1.25
G6PC3	Glucose 6 Phosphatase, Catalytic, 3	0.010	1.25
ARRDC4	Arrestin Domain Containing 4	0.027	1.25

ARAP1	Arfgap With Rhogap Domain, Ankyrin Repeat And PH Domain 1	0.001	1.25
HIST1H2BL	Histone Cluster 1, H2bl	0.015	1.24
TRAJ25	T Cell Receptor Alpha Joining 25 (Non-Functional)	0.037	1.24
CNP	2',3'-Cyclic Nucleotide 3' Phosphodiesterase	< 0.001	1.24
SNRPN	Small Nuclear Ribonucleoprotein Polypeptide N	0.036	1.24
HSP90B1	Heat Shock Protein 90kda Beta (Grp94), Member 1	0.019	1.24
C10orf54	Chromosome 10 Open Reading Frame 54	0.039	1.24
TBC1D3H	TBC1 Domain Family, Member 3H	0.021	1.24
PRIC285	Peroxisomal Proliferator-Activated Receptor A	0.015	1.24
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme, Catalytic Polypeptide-Li	0.025	1.24
IGLV2-18	Immunoglobulin Lambda Variable 2-18	0.047	1.23
GANAB	Glucosidase, Alpha; Neutral AB	0.031	1.23
HIST1H1E	Histone Cluster 1, H1e	0.002	1.23
IGLV1-44	Immunoglobulin Lambda Variable 1-44	0.023	1.23
CRISP3	Cysteine-Rich Secretory Protein 3	0.044	1.23
AP5B1	Adaptor-Related Protein Complex 5, Beta 1 Subunit	0.026	1.23
C2CD2	C2 Calcium-Dependent Domain Containing 2	0.026	1.23
JAK3	Janus Kinase 3	0.024	1.23
PLCG2	Phospholipase C, Gamma 2 (Phosphatidylinositol-Specific)	0.026	1.23
U2AF2	U2 Small Nuclear RNA Auxiliary Factor 2	0.030	1.23
FCAR	Fc Fragment Of Iga, Receptor	0.018	1.23
FMNL1	Formin-Like 1	0.047	1.23
DUS3L	Dihydrouridine Synthase 3-Like (S. Cerevisiae)	0.031	1.22
RFNG	RFNG O-Fucosylpeptide 3-Beta-N-Acetylglucosaminyltransferase	0.019	1.22
IGK@	Immunoglobulin Kappa Locus	0.041	1.22
NBEAL2	Neurobeachin-Like 2	0.004	1.22
DNASE1	Deoxyribonuclease I	0.043	1.22
TBC1D3F	TBC1 Domain Family, Member 3F	0.012	1.22
SNORA75	Small Nucleolar RNA, H/ACA Box 75	0.028	1.22

HIST1H3B	Histone Cluster 1, H3b	0.023	1.22
STAT2	Signal Transducer And Activator Of Transcription 2, 113kda	0.049	1.21
MAN2B1	Mannosidase, Alpha, Class 2B, Member 1	0.032	1.21
PKN1	Protein Kinase N1	0.021	1.21
TKT	Transketolase	0.004	1.21
HCFC1	Host Cell Factor C1 (VP16-Accessory Protein)	0.028	1.21
HIST1H2BC	Histone Cluster 1, H2bc	0.038	1.21
ZNF646	Zinc Finger Protein 646	0.018	1.21
CLCN7	Chloride Channel, Voltage-Sensitive 7	0.036	1.21
IGKV4-1	Immunoglobulin Kappa Variable 4-1	0.013	1.21
DISC1-IT1	DISC1 Intronic Transcript 1 (Non-Protein Coding)	0.019	1.21
ADRBK1	Adrenergic, Beta, Receptor Kinase 1	0.006	1.21
TET3	Tet Methylcytosine Dioxygenase 3	0.022	1.21
SNORA45	Small Nucleolar RNA, H/ACA Box 45	0.032	1.21
ITGAX	Integrin, Alpha X (Complement Component 3 Receptor 4 Subunit)	0.034	1.20
NLRC5	NLR Family, CARD Domain Containing 5	0.006	1.20
RGS19	Regulator Of G-Protein Signaling 19	0.010	1.20
STOM	Stomatin	0.005	1.20
MAPK8IP3	Mitogen-Activated Protein Kinase 8 Interacting Protein 3	0.010	1.20
TRANK1	Tetratricopeptide Repeat And Ankyrin Repeat Containing 1	0.038	1.20
PNPLA6	Patatin-Like Phospholipase Domain Containing 6	0.016	1.20
SPATA25	Spermatogenesis Associated 25	0.024	-1.20
RPL13AP20	Ribosomal Protein L13a Pseudogene 20	0.020	-1.20
RAB11B-AS1	RAB11B Antisense RNA 1 (Non-Protein Coding)	0.021	-1.21
FLJ43681	Ribosomal Protein L23a Pseudogene	0.049	-1.21
TMEM220	Transmembrane Protein 220	0.005	-1.21
IGLV4-60	Immunoglobulin Lambda Variable 4-60	0.028	-1.21
ZNRF2P1	Zinc And Ring Finger 2 Pseudogene 1	0.031	-1.21

TIGD3	Tigger Transposable Element Derived 3	0.015	-1.22
TXNDC17	Thioredoxin Domain Containing 17	0.046	-1.23
KRTAP20-1	Keratin Associated Protein 20-1	0.002	-1.24
CBR3	Carbonyl Reductase 3	0.032	-1.25
TMSB4XP4	Thymosin Beta 4, X-Linked Pseudogene 4	0.035	-1.25
HCG8	HLA Complex Group 8	0.033	-1.26
SHFM1	Split Hand/Foot Malformation (Ectrodactyly) Type 1	0.001	-1.26
KLHL22-IT1	KLHL22 Intronic Transcript 1 (Non-Protein Coding)	0.031	-1.26
TRAV41	T Cell Receptor Alpha Variable 41	0.007	-1.27
POLR2J	Polymerase (RNA) II (DNA Directed) Polypeptide J, 13.3kda	0.005	-1.28
LINC00243	Long Intergenic Non-Protein Coding RNA 243	0.043	-1.29
SPINK8	Serine Peptidase Inhibitor, Kazal Type 8 (Putative)	0.034	-1.30
PIN1P1	Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 1 Pseudogen	0.002	-1.31
SLC9A9-AS2	SLC9A9 Antisense RNA 2 (Non-Protein Coding)	0.013	-1.32
SNORA11	Small Nucleolar RNA, H/ACA Box 11	0.019	-1.37
LHB	Luteinizing Hormone Beta Polypeptide	0.033	-1.43
AHSP	Alpha Hemoglobin Stabilizing Protein	0.028	-1.45
SNORD93	Small Nucleolar RNA, C/D Box 93	0.027	-1.64
TUBB2A	Tubulin, Beta 2A Class Iia	0.041	-1.75

Table 6D. List of 23 genes differently modulated in males HR vs males LR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TCL1B	T-Cell Leukemia/Lymphoma 1B	0.030	1.42
SNORA45	Small Nucleolar RNA, H/ACA Box 45	< 0.001	1.37
SNORD14A	Small Nucleolar RNA, C/D Box 14A	0.021	1.37
TRAJ20	T Cell Receptor Alpha Joining 20	0.011	1.34
TRAV29DV5	T Cell Receptor Alpha Variable 29/Delta Variable 5	0.013	1.24
RN5S104	RNA, 5S Ribosomal 104	0.021	1.20
KRTAP9-8	Keratin Associated Protein 9-8	0.006	-1.21
SEC14L1P1	SEC14-Like 1 Pseudogene 1	0.014	-1.21
RN5S370	RNA, 5S Ribosomal 370	0.032	-1.21
TRAV41	T Cell Receptor Alpha Variable 41	0.028	-1.21
ZNF718	Zinc Finger Protein 718	0.010	-1.22
ZNF486	Zinc Finger Protein 486	0.026	-1.22
MAP3K11	Mitogen-Activated Protein Kinase Kinase Kinase 11	0.048	-1.23
RPL23AP53	Ribosomal Protein L23a Pseudogene 53	0.027	-1.23
RN5S203	RNA, 5S Ribosomal 203	0.049	-1.24
LILRA6	Leukocyte Immunoglobulin-Like Receptor, Subfamily A	0.044	-1.26
RAVER2	Ribonucleoprotein, PTB-Binding 2	0.008	-1.26
RNY4P19	RNA, Ro-Associated Y4 Pseudogene 19	0.042	-1.26
ANAPC1	Anaphase Promoting Complex Subunit 1	0.048	-1.28
RNU5E-9P	RNA, U5E Small Nuclear 9, Pseudogene	0.042	-1.28
P2RX1	Purinergic Receptor P2X, Ligand-Gated Ion Channel, 1	0.018	-1.30
FAR1-IT1	FAR1 Intronic Transcript 1 (Non-Protein Coding)	0.005	-1.38
SIGLEC14	Sialic Acid Binding Ig-Like Lectin 14	0.047	-1.47

Table 7D. List of 42 genes differently modulated in females MDD vs females HR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
MX1	Myxovirus (Influenza Virus) Resistance 1, Interferon-Inducible P	0.007	1.50
TRAJ19	T Cell Receptor Alpha Joining 19 (Non-Functional)	0.043	1.47
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.023	1.47
TRAJ40	T Cell Receptor Alpha Joining 40	0.049	1.45
PLGLB1	Plasminogen-Like B1	0.037	1.41
SNORA36B	Small Nucleolar RNA, H/ACA Box 36B	0.030	1.39
ANO7L1	Anoctamin 7-Like 1	0.010	1.38
TNFAIP6	Tumor Necrosis Factor, Alpha-Induced Protein 6	0.025	1.38
HP	Haptoglobin	0.031	1.38
CD177	CD177 Molecule	0.002	1.37
IFIT2	Interferon-Induced Protein With Tetratricopeptide Repeats 2	0.014	1.35
FAM106CP	Family With Sequence Similarity 106, Member C, Pseudogene	0.018	1.34
SLED1	Proteoglycan 3 Pseudogene	0.037	1.33
HPSE	Heparanase	0.009	1.30
XRRA1	X-Ray Radiation Resistance Associated 1	0.025	1.30
SNORD99	Small Nucleolar RNA, C/D Box 99	0.034	1.30
RTP4	Receptor (Chemosensory) Transporter Protein 4	0.039	1.27
LINC00264	Long Intergenic Non-Protein Coding RNA 264	0.033	1.26
C19orf59	Chromosome 19 Open Reading Frame 59	0.047	1.25
GBP4	Guanylate Binding Protein 4	0.030	1.25
NEBL	Nebulette	0.012	1.25
GK	Glycerol Kinase	0.021	1.25
SPATS2L	Spermatogenesis Associated, Serine-Rich 2-Like	0.014	1.25
IL8	Interleukin 8	0.019	1.24
STAT2	Signal Transducer And Activator Of Transcription 2, 113kda	0.035	1.23
PNPT1	Polyribonucleotide Nucleotidyltransferase 1	0.045	1.22

CCZ1B	CCZ1 Vacuolar Protein Trafficking And Biogenesis Associated	0.003	1.22
CEACAM3	Carcinoembryonic Antigen-Related Cell Adhesion Molecule 3	0.022	1.20
ZCCHC2	Zinc Finger, CCHC Domain Containing 2	0.031	1.20
DHX58	DEXH (Asp-Glu-X-His) Box Polypeptide 58	0.044	1.20
KCNRG	Potassium Channel Regulator	0.012	-1.20
RNF144B	Ring Finger Protein 144B	0.048	-1.21
IGHV6-1	Immunoglobulin Heavy Variable 6-1	0.012	-1.21
RPL19P12	Ribosomal Protein L19 Pseudogene 12	0.031	-1.21
HIST1H3J	Histone Cluster 1, H3j	< 0.001	-1.21
ZNF33B	Zinc Finger Protein 33B	0.007	-1.23
ADAM6	ADAM Metallopeptidase Domain 6 (Pseudogene)	0.048	-1.24
HRASLS5	HRAS-Like Suppressor Family, Member 5	0.013	-1.24
OR2T34	Olfactory Receptor, Family 2, Subfamily T, Member 34	< 0.001	-1.25
TNNT1	Troponin T Type 1 (Skeletal, Slow)	0.036	-1.28
TRAV23DV6	T Cell Receptor Alpha Variable 23/Delta Variable 6	0.001	-1.31
C4BPA	Complement Component 4 Binding Protein, Alpha	0.031	-1.60

Table 8D. List of 67 genes differently modulated in females MDD vs females LR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
IFI44L	Interferon-Induced Protein 44-Like	0.035	1.75
RSAD2	Radical S-Adenosyl Methionine Domain Containing 2	0.011	1.71
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.004	1.63
IFIT1	Interferon-Induced Protein With Tetratricopeptide Repeats 1	0.021	1.59
CASP5	Caspase 5, Apoptosis-Related Cysteine Peptidase	0.013	1.52
PLGLB1	Plasminogen-Like B1	0.018	1.48
CMPK2	Cytidine Monophosphate (UMP-CMP) Kinase 2, Mitochondrial	0.030	1.46
MX1	Myxovirus (Influenza Virus) Resistance 1, Interferon-Inducible P	0.013	1.45
IFIT2	Interferon-Induced Protein With Tetratricopeptide Repeats 2	0.006	1.40
CARD17	Caspase Recruitment Domain Family, Member 17	0.023	1.37
TNFAIP6	Tumor Necrosis Factor, Alpha-Induced Protein 6	0.033	1.36
HPSE	Heparanase	0.003	1.36
EIF2AK2	Eukaryotic Translation Initiation Factor 2-Alpha Kinase 2	0.016	1.34
SLED1	Proteoglycan 3 Pseudogene	0.041	1.32
PNPT1	Polyribonucleotide Nucleotidyltransferase 1	0.006	1.32
SNORA16B	Small Nucleolar RNA, H/ACA Box 16B	0.013	1.32
IFIT5	Interferon-Induced Protein With Tetratricopeptide Repeats 5	0.022	1.31
CEP19	Centrosomal Protein 19kda	0.006	1.31
KCNJ2-AS1	KCNJ2 Antisense RNA 1	0.011	1.29
SPATS2L	Spermatogenesis Associated, Serine-Rich 2-Like	0.006	1.28
IFIH1	Interferon Induced With Helicase C Domain 1	0.038	1.27
SAMD9L	Sterile Alpha Motif Domain Containing 9-Like	0.030	1.27
AIM2	Absent In Melanoma 2	0.026	1.27
EAF1-AS1	EAF1 Antisense RNA 1	0.043	1.27
IFITM3	Interferon Induced Transmembrane Protein 3	0.041	1.27
HERC6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family	0.030	1.26

SLC26A8	Solute Carrier Family 26, Member 8	0.024	1.25
DPRXP4	Divergent-Paired Related Homeobox Pseudogene 4	0.032	1.25
FFAR2	Free Fatty Acid Receptor 2	0.004	1.24
MORC3	MORC Family CW-Type Zinc Finger 3	0.009	1.24
CD177	CD177 Molecule	0.027	1.24
FAR2	Fatty Acyl Coa Reductase 2	0.014	1.23
NSFP1	N-Ethylmaleimide-Sensitive Factor Pseudogene 1	0.023	1.23
DOCK4	Dedicator Of Cytokinesis 4	0.038	1.23
TDRD7	Tudor Domain Containing 7	0.002	1.22
USP32P2	Ubiquitin Specific Peptidase 32 Pseudogene 2	0.004	1.22
DDX58	DEAD (Asp-Glu-Ala-Asp) Box Polypeptide 58	0.024	1.22
ZCCHC2	Zinc Finger, CCHC Domain Containing 2	0.023	1.22
TLR2	Toll-Like Receptor 2	0.005	1.21
NAPG	N-Ethylmaleimide-Sensitive Factor Attachment Protein, Gamma	< 0.001	1.21
HIST1H2BC	Histone Cluster 1, H2bc	0.004	1.21
CAPNS2	Calpain, Small Subunit 2	0.003	1.21
LPP-AS2	LPP Antisense RNA 2	0.037	1.20
GBP3	Guanylate Binding Protein 3	0.044	1.20
TECPR2	Tectonin Beta-Propeller Repeat Containing 2	0.023	1.20
SAMD9	Sterile Alpha Motif Domain Containing 9	0.037	1.20
ZNF33B	Zinc Finger Protein 33B	0.019	-1.20
NEFL	Neurofilament, Light Polypeptide	0.003	-1.20
SH3GL1	SH3-Domain GRB2-Like 1	0.008	-1.20
TPTE	Transmembrane Phosphatase With Tensin Homology	0.003	-1.20
CTSW	Cathepsin W	0.032	-1.21
TBC1D22B	TBC1 Domain Family, Member 22B	0.048	-1.21
FAM106A	Family With Sequence Similarity 106, Member A	0.002	-1.22
PVALB	Parvalbumin	0.048	-1.22
SNORD92	Small Nucleolar RNA, C/D Box 92	0.003	-1.23

ZNF17	Zinc Finger Protein 17	0.003	-1.23
SLC35E2	Solute Carrier Family 35, Member E2	0.022	-1.25
ADAM6	ADAM Metallopeptidase Domain 6	0.042	-1.25
CBR3	Carbonyl Reductase 3	0.029	-1.26
SNORD10	Small Nucleolar RNA, C/D Box 10	0.020	-1.27
CATSPER2 P1	Cation Channel, Sperm Associated 2 Pseudogene 1	0.021	-1.27
PRSS21	Protease, Serine, 21 (Testisin)	0.031	-1.28
SNORA84	Small Nucleolar RNA, H/ACA Box 84	0.047	-1.28
CCDC144A	Coiled-Coil Domain Containing 144A	0.044	-1.36
SMYD3-IT1	SMYD3 Intronic Transcript 1	0.014	-1.44
TUBBP5	Tubulin, Beta Pseudogene 5	0.023	-1.49
C4BPA	Complement Component 4 Binding Protein, Alpha	0.021	-1.66

Table 9D. List of 43 genes differently modulated in females HR vs females LR in the microarray analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
MORC3	MORC Family CW-Type Zinc Finger 3	0.001	1.33
TRAJ50	T Cell Receptor Alpha Joining 50	0.048	1.31
IGHV1-3	Immunoglobulin Heavy Variable 1-3	0.042	1.29
NSFP1	N-Ethylmaleimide-Sensitive Factor Pseudogene 1	0.007	1.28
DHFR	Dihydrofolate Reductase	0.039	1.26
SNORA70G	Small Nucleolar RNA, H/ACA Box 70G	0.026	1.22
DRG1	Developmentally Regulated GTP Binding Protein 1	0.046	1.21
CEP19	Centrosomal Protein 19kda	0.048	1.21
FAM215A	Family With Sequence Similarity 215, Member A	0.032	-1.20
PLVAP	Plasmalemma Vesicle Associated Protein	0.049	-1.21
GPR146	G Protein-Coupled Receptor 146	0.021	-1.21
March2	Membrane-Associated Ring Finger (C3HC4) 2, E3 Ubiquitin Protein	0.014	-1.21
FMO4	Flavin Containing Monooxygenase 4	0.002	-1.22
GBAP1	Glucosidase, Beta, Acid Pseudogene 1	< 0.001	-1.22
GRIK1-AS2	GRIK1 Antisense RNA 2 (Non-Protein Coding)	0.024	-1.22
MAP2K3	Mitogen-Activated Protein Kinase Kinase 3	0.012	-1.22
MS4A7	Membrane-Spanning 4-Domains, Subfamily A, Member 7	0.026	-1.22
LPAL2	Lipoprotein, Lp(A)-Like 2, Pseudogene	0.028	-1.23
BABAM1	BRISC And BRCA1 A Complex Member 1	0.050	-1.25
CATSPER2P1	Cation Channel, Sperm Associated 2 Pseudogene 1	0.033	-1.25
SLC35E2	Solute Carrier Family 35, Member E2	0.020	-1.25
PIM1	Pim-1 Oncogene	0.043	-1.25
ZNF14	Zinc Finger Protein 14	0.008	-1.25
FAM210B	Family With Sequence Similarity 210, Member B	0.032	-1.27

CTSW	Cathepsin W	0.008	-1.27
TRIM58	Tripartite Motif Containing 58	0.038	-1.27
OSBP2	Oxysterol Binding Protein 2	0.010	-1.27
BSG	Basigin (Ok Blood Group)	0.020	-1.27
SNAR-D	Small ILF3/NF90-Associated RNA D	0.030	-1.27
CRYBB2P1	Crystallin, Beta B2 Pseudogene 1	0.042	-1.28
March8	Membrane-Associated Ring Finger (C3HC4) 8, E3 Ubiquitin Prote	0.007	-1.28
SMOX	Spermine Oxidase	0.015	-1.28
EPB49	Erythrocyte Membrane Protein Band 4.9 (Dematin)	0.033	-1.30
FAM106CP	Family With Sequence Similarity 106, Member C, Pseudogene	0.028	-1.32
MMP8	Matrix Metallopeptidase 8 (Neutrophil Collagenase)	0.027	-1.33
EPB42	Erythrocyte Membrane Protein Band 4.2	0.041	-1.34
SH2D1B	SH2 Domain Containing 1B	0.013	-1.34
C19orf77	Chromosome 19 Open Reading Frame 77	0.034	-1.36
OR2W3	Olfactory Receptor, Family 2, Subfamily W, Member 3	0.007	-1.40
RETN	Resistin	0.015	-1.40
KIR3DL2	Killer Cell Immunoglobulin-Like Receptor	0.018	-1.45
CCDC144A	Coiled-Coil Domain Containing 144A	0.010	-1.48
PDIA3P	Protein Disulfide Isomerase Family A, Member 3 Pseudogene	0.003	-1.63

APPENDIX E

Table 1E. List of 313 genes differently modulated in MDD vs HR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3G	TBC1 Domain Family Member 3G	0.007	279.44
TBC1D3	TBC1 Domain Family Member 3	0.001	8.96
PTP4A1	Protein Tyrosine Phosphatase 4A1	0.011	4.71
ADARB2	Adenosine Deaminase RNA Specific B2 (Inactive)	0.001	4.59
KLHL41	Kelch Like Family Member 41	< 0.001	3.15
CD177	CD177 Molecule	< 0.001	2.76
CCL8	C-C Motif Chemokine Ligand 8	0.010	2.32
NDST3	N-Deacetylase And N-Sulfotransferase 3	0.010	2.30
RSAD2	Radical S-Adenosyl Methionine Domain Containing 2	0.001	2.18
DCN	Decorin	0.002	2.18
IFI44L	Interferon Induced Protein 44 Like	0.001	2.13
DOK6	Docking Protein 6	0.011	2.09
KLF14	Kruppel Like Factor 14	0.017	2.08
POC1B-GALNT4	POC1B-GALNT4 Readthrough	0.045	2.07
NEBL	Nebulette	0.037	2.03
SYCE1	Synaptonemal Complex Central Element Protein 1	0.027	2.00
MRGPRE	MAS Related GPR Family Member E	0.025	1.99
CCL2	C-C Motif Chemokine Ligand 2	0.007	1.99
EPB41L4B	Erythrocyte Membrane Protein Band 4.1 Like 4B	0.027	1.99
ITGA8	Integrin Subunit Alpha 8	0.021	1.97
ZBED9	Zinc Finger BED-Type Containing 9	0.023	1.96
PLSCR2	Phospholipid Scramblase 2	0.023	1.95
TEAD1	TEA Domain Transcription Factor 1	0.036	1.95

SIGLEC1	Sialic Acid Binding Ig Like Lectin 1	0.002	1.94
NEUROD4	Neuronal Differentiation 4	0.028	1.93
GPR26	G Protein-Coupled Receptor 26	0.040	1.92
RGS13	Regulator Of G Protein Signaling 13	0.027	1.88
CD200R1L	CD200 Receptor 1 Like	0.014	1.87
CCL25	C-C Motif Chemokine Ligand 25	0.011	1.87
IFIT1	Interferon Induced Protein With Tetratricopeptide Repeats 1	0.003	1.85
IFI27	Interferon Alpha Inducible Protein 27	0.020	1.84
ISG15	ISG15 Ubiquitin Like Modifier	0.002	1.83
SHISA3	Shisa Family Member 3	0.037	1.79
CMPK2	Cytidine/Uridine Monophosphate Kinase 2	0.004	1.76
PSMB11	Proteasome Subunit Beta 11	0.015	1.76
USP18	Ubiquitin Specific Peptidase 18	0.001	1.74
PARD3B	Par-3 Family Cell Polarity Regulator Beta	0.023	1.74
COL1A2	Collagen Type I Alpha 2 Chain	0.020	1.74
FBLIM1	Filamin Binding LIM Protein 1	0.045	1.74
CD163L1	CD163 Molecule Like 1	0.024	1.73
IFI44	Interferon Induced Protein 44	0.005	1.72
OAS3	2'-5'-Oligoadenylate Synthetase 3	0.005	1.70
DLC1	DLC1 Rho Gtpase Activating Protein	0.006	1.69
APOBEC3B	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3B	0.012	1.68
DGKI	Diacylglycerol Kinase Iota	0.043	1.67
TERT	Telomerase Reverse Transcriptase	0.006	1.66
POU5F2	POU Domain Class 5, Transcription Factor 2	0.018	1.63
HTRA3	Htra Serine Peptidase 3	0.019	1.63
MX1	MX Dynamin Like Gtpase 1	0.003	1.62
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.005	1.62
DZIP1L	DAZ Interacting Zinc Finger Protein 1 Like	0.005	1.61
PRRG1	Proline Rich And Gla Domain 1	0.037	1.61

LOC112267968	Uncharacterized LOC112267968	0.018	1.59
NACAD	NAC Alpha Domain Containing	0.030	1.58
PDE3A	Phosphodiesterase 3A	0.024	1.54
IFI6	Interferon Alpha Inducible Protein 6	0.009	1.54
SPATA1	Spermatogenesis Associated 1	0.007	1.54
IFIT3	Interferon Induced Protein With Tetratricopeptide Repeats 3	0.012	1.53
CXCL10	C-X-C Motif Chemokine Ligand 10	0.029	1.53
KCNMA1	Potassium Calcium-Activated Channel Subfamily M Alpha 1	0.020	1.53
PADI6	Peptidyl Arginine Deiminase 6	0.013	1.53
C1QC	Complement C1q C Chain	0.049	1.53
TRIM6	Tripartite Motif Containing 6	0.011	1.52
C1QB	Complement C1q B Chain	0.036	1.50
PPARG	Peroxisome Proliferator Activated Receptor Gamma	0.025	1.50
MCM10	Minichromosome Maintenance 10 Replication Initiation Factor	0.013	1.49
MMP8	Matrix Metallopeptidase 8	0.021	1.49
OAS1	2'-5'-Oligoadenylate Synthetase 1	0.008	1.48
SLC41A2	Solute Carrier Family 41 Member 2	0.011	1.48
CDC25A	Cell Division Cycle 25A	0.008	1.48
RNF43	Ring Finger Protein 43	0.004	1.47
LIPH	Lipase H	0.048	1.47
A3GALT2	Alpha 1,3-Galactosyltransferase 2	0.035	1.46
KNL1	Kinetochore Scaffold 1	0.010	1.46
HPN	Hepsin	0.023	1.46
AGRN	Agrin	0.010	1.45
EXOC3L1	Exocyst Complex Component 3 Like 1	0.019	1.45
LAMP3	Lysosomal Associated Membrane Protein 3	0.040	1.45
IFIT2	Interferon Induced Protein With Tetratricopeptide Repeats 2	0.008	1.45
MYH11	Myosin Heavy Chain 11	0.027	1.44
OASL	2'-5'-Oligoadenylate Synthetase Like	0.020	1.43

SAMD4A	Sterile Alpha Motif Domain Containing 4A	0.005	1.43
PRG4	Proteoglycan 4	0.005	1.43
LGALS9B	Galectin 9B	0.008	1.43
HEY1	Hes Related Family Bhlh Transcription Factor With YRPW Motif 1	0.017	1.43
RSPH9	Radial Spoke Head Component 9	0.015	1.42
MSR1	Macrophage Scavenger Receptor 1	0.019	1.41
FRMD3	FERM Domain Containing 3	0.001	1.41
CHRFAM7A	CHRNA7 (Exons 5-10) And FAM7A (Exons A-E) Fusion	0.018	1.41
CCDC194	Coiled-Coil Domain Containing 194	0.033	1.41
MIXL1	Mix Paired-Like Homeobox	0.038	1.40
CCNA1	Cyclin A1	0.024	1.40
AFAP1L1	Actin Filament Associated Protein 1 Like 1	0.033	1.40
TMC4	Transmembrane Channel Like 4	0.005	1.40
OAS2	2'-5'-Oligoadenylate Synthetase 2	0.013	1.40
C1QA	Complement C1q A Chain	0.028	1.40
NEXN	Nexilin F-Actin Binding Protein	0.016	1.39
CA6	Carbonic Anhydrase 6	0.050	1.39
SOX7	SRY-Box Transcription Factor 7	0.005	1.39
SERPINE1	Serpin Family E Member 1	0.003	1.39
ANO5	Anoctamin 5	0.022	1.39
ARHGAP23	Rho Gtpase Activating Protein 23	0.024	1.39
ARNTL2	Aryl Hydrocarbon Receptor Nuclear Translocator Like 2	0.021	1.38
CIBAR1	CBY1 Interacting BAR Domain Containing 1	0.047	1.38
GLDC	Glycine Decarboxylase	0.046	1.37
SAPCD1	Suppressor APC Domain Containing 1	0.020	1.36
TNFAIP6	TNF Alpha Induced Protein 6	0.026	1.36
KIF4A	Kinesin Family Member 4A	0.030	1.36
TBC1D8B	TBC1 Domain Family Member 8B	0.020	1.36
EDA	Ectodysplasin A	0.018	1.36

ERG	ETS Transcription Factor ERG	0.041	1.35
MARCO	Macrophage Receptor With Collagenous Structure	0.013	1.35
EIF2AK2	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2	0.011	1.34
PRSS21	Serine Protease 21	0.021	1.34
UNC13B	Unc-13 Homolog B	0.001	1.33
ASPM	Assembly Factor For Spindle Microtubules	0.040	1.33
HERC6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6	0.005	1.33
TDRP	Testis Development Related Protein	0.023	1.33
HMMR	Hyaluronan Mediated Motility Receptor	0.046	1.33
FAM187A	Family With Sequence Similarity 187 Member A	0.047	1.32
ANGPT1	Angiopoietin 1	0.027	1.32
LOXHD1	Lipoxygenase Homology PLAT Domains 1	0.025	1.32
CD80	CD80 Molecule	0.028	1.32
IFITM3	Interferon Induced Transmembrane Protein 3	0.048	1.32
PLSCR1	Phospholipid Scramblase 1	0.018	1.32
CD1B	CD1b Molecule	0.038	1.32
DDX60	Dexd/H-Box Helicase 60	0.018	1.31
SH3PXD2B	SH3 And PX Domains 2B	0.039	1.31
TMEM51	Transmembrane Protein 51	0.024	1.31
HP	Haptoglobin	0.028	1.31
RGS16	Regulator Of G Protein Signaling 16	0.003	1.31
IFIT5	Interferon Induced Protein With Tetratricopeptide Repeats 5	0.018	1.30
TMEM40	Transmembrane Protein 40	0.046	1.30
CCNE2	Cyclin E2	0.030	1.30
ZNF107	Zinc Finger Protein 107	0.002	1.30
TLR3	Toll Like Receptor 3	0.024	1.30
GNAI1	G Protein Subunit Alpha I1	0.032	1.29
SUCNR1	Succinate Receptor 1	0.040	1.29
BAMBI	BMP And Activin Membrane Bound Inhibitor	0.045	1.28

IFIH1	Interferon Induced With Helicase C Domain 1	0.010	1.28
HOXA9	Homeobox A9	0.036	1.28
RECQL4	Recq Like Helicase 4	0.009	1.28
RIPOR3	RIPOR Family Member 3	0.027	1.28
MT2A	Metallothionein 2A	0.043	1.28
GPER1	G Protein-Coupled Estrogen Receptor 1	0.030	1.27
COL4A4	Collagen Type IV Alpha 4 Chain	0.024	1.27
EME1	Essential Meiotic Structure-Specific Endonuclease 1	0.027	1.27
CLDN12	Claudin 12	0.034	1.27
TNS4	Tensin 4	0.050	1.26
TENM1	Teneurin Transmembrane Protein 1	0.033	1.26
CBX2	Chromobox 2	0.021	1.26
ANGPTL6	Angiopoietin Like 6	0.023	1.26
ZNF684	Zinc Finger Protein 684	0.028	1.25
ANKRD9	Ankyrin Repeat Domain 9	0.016	1.25
PGAM2	Phosphoglycerate Mutase 2	0.037	1.25
DDX60L	Dexd/H-Box 60 Like	0.024	1.24
ICA1L	Islet Cell Autoantigen 1 Like	0.026	1.24
CDCA7	Cell Division Cycle Associated 7	0.015	1.24
TMEM255B	Transmembrane Protein 255B	0.045	1.24
DHX58	Dexh-Box Helicase 58	0.028	1.24
RAD9B	RAD9 Checkpoint Clamp Component B	0.034	1.23
LRRCC1	Leucine Rich Repeat And Coiled-Coil Centrosomal Protein 1	0.031	1.23
BMP6	Bone Morphogenetic Protein 6	0.002	1.23
TFEC	Transcription Factor EC	0.023	1.23
KIF24	Kinesin Family Member 24	0.020	1.23
TNFSF10	TNF Superfamily Member 10	0.025	1.22
SLC16A7	Solute Carrier Family 16 Member 7	0.010	1.22
AGBL2	AGBL Carboxypeptidase 2	0.024	1.22
CDKL1	Cyclin Dependent Kinase Like 1	0.036	1.22

CCDC62	Coiled-Coil Domain Containing 62	0.047	1.21
NOMO3	NODAL Modulator 3	0.003	1.20
INSL3	Insulin Like 3	0.034	1.20
NT5C3A	5'-Nucleotidase, Cytosolic IIIA	0.013	1.20
LRP11	LDL Receptor Related Protein 11	0.036	-1.21
PDE7B	Phosphodiesterase 7B	0.008	-1.21
RPL11	Ribosomal Protein L11	0.046	-1.21
ATP1A3	Atpase Na+/K+ Transporting Subunit Alpha 3	0.030	-1.21
CYP4F22	Cytochrome P450 Family 4 Subfamily F Member 22	0.042	-1.22
EEF1B2	Eukaryotic Translation Elongation Factor 1 Beta 2	0.034	-1.23
TRIP10	Thyroid Hormone Receptor Interactor 10	0.007	-1.23
MMP23B	Matrix Metallopeptidase 23B	0.029	-1.23
PPP1R13L	Protein Phosphatase 1 Regulatory Subunit 13 Like	0.001	-1.25
NEFL	Neurofilament Light Chain	0.013	-1.26
BFSP1	Beaded Filament Structural Protein 1	0.034	-1.27
ATP2A1	Atpase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 1	0.043	-1.27
GCAT	Glycine C-Acetyltransferase	0.043	-1.28
CUZD1	CUB And Zona Pellucida Like Domains 1	0.049	-1.28
GLB1L2	Galactosidase Beta 1 Like 2	0.048	-1.28
DRICH1	Aspartate Rich 1	0.021	-1.29
LRRC36	Leucine Rich Repeat Containing 36	0.046	-1.29
TMSB15B	Thymosin Beta 15B	0.043	-1.29
TFCP2L1	Transcription Factor CP2 Like 1	0.035	-1.29
CBR3	Carbonyl Reductase 3	0.005	-1.29
ZNF683	Zinc Finger Protein 683	0.019	-1.30
SYBU	Syntabulin	0.021	-1.30
REELD1	Reeler Domain Containing 1	0.028	-1.30
GSTA4	Glutathione S-Transferase Alpha 4	0.004	-1.30
SNX32	Sorting Nexin 32	0.015	-1.31

GARNL3	Gtpase Activating Rap/Rangap Domain Like 3	0.036	-1.32
TTC39A	Tetratricopeptide Repeat Domain 39A	0.021	-1.32
SHD	Src Homology 2 Domain Containing Transforming Protein D	0.049	-1.32
PGLYRP2	Peptidoglycan Recognition Protein 2	0.030	-1.32
COLEC12	Collectin Subfamily Member 12	0.033	-1.33
EPHB3	EPH Receptor B3	0.017	-1.33
CEMP1	Cementum Protein 1	0.031	-1.33
PFDN4	Prefoldin Subunit 4	0.034	-1.33
TNNT1	Troponin T1, Slow Skeletal Type	0.035	-1.34
ARHGEF25	Rho Guanine Nucleotide Exchange Factor 25	0.010	-1.34
PLA2G2D	Phospholipase A2 Group IID	0.011	-1.34
LRRN2	Leucine Rich Repeat Neuronal 2	0.042	-1.34
LBX2	Ladybird Homeobox 2	0.037	-1.34
PMFBP1	Polyamine Modulated Factor 1 Binding Protein 1	0.025	-1.34
RHCE	Rh Blood Group Ccee Antigens	0.021	-1.34
CYP46A1	Cytochrome P450 Family 46 Subfamily A Member 1	0.006	-1.35
ZDHHC11B	Zinc Finger DHHC-Type Containing 11B	0.018	-1.35
SEZ6L2	Seizure Related 6 Homolog Like 2	0.044	-1.35
FAM110C	Family With Sequence Similarity 110 Member C	0.035	-1.36
PRR36	Proline Rich 36	0.049	-1.36
TLCD1	TLC Domain Containing 1	0.029	-1.36
GRIK5	Glutamate Ionotropic Receptor Kainate Type Subunit 5	0.008	-1.36
SFRP5	Secreted Frizzled Related Protein 5	0.047	-1.37
IGDCC4	Immunoglobulin Superfamily DCC Subclass Member 4	0.014	-1.37
PLA2G4C	Phospholipase A2 Group IVC	0.036	-1.37
MOCS1	Molybdenum Cofactor Synthesis 1	0.017	-1.38
CCDC171	Coiled-Coil Domain Containing 171	0.042	-1.39
GDF7	Growth Differentiation Factor 7	0.025	-1.39
PLLP	Plasmolipin	< 0.001	-1.39
MARVELD2	MARVEL Domain Containing 2	0.031	-1.39

BICDL2	BICD Family Like Cargo Adaptor 2	0.047	-1.40
SPTSSB	Serine Palmitoyltransferase Small Subunit B	0.044	-1.40
PRODH	Proline Dehydrogenase 1	0.031	-1.40
EFNA5	Ephrin A5	0.043	-1.40
RPL9	Ribosomal Protein L9	0.010	-1.41
COL15A1	Collagen Type XV Alpha 1 Chain	0.045	-1.41
MAST1	Microtubule Associated Serine/Threonine Kinase 1	0.024	-1.41
PLS3	Plastin 3	0.009	-1.42
TCTE1	T-Complex-Associated-Testis-Expressed 1	0.043	-1.42
CCDC163	Coiled-Coil Domain Containing 163	0.010	-1.42
KNDC1	Kinase Non-Catalytic C-Lobe Domain Containing 1	0.003	-1.42
ABCA6	ATP Binding Cassette Subfamily A Member 6	0.015	-1.43
FHAD1	Forkhead Associated Phosphopeptide Binding Domain 1	0.033	-1.43
PTPRG	Protein Tyrosine Phosphatase Receptor Type G	0.024	-1.44
ANKRD53	Ankyrin Repeat Domain 53	0.045	-1.44
SPP1	Secreted Phosphoprotein 1	0.034	-1.44
ATP2B3	Atpase Plasma Membrane Ca2+ Transporting 3	0.050	-1.45
GRM7	Glutamate Metabotropic Receptor 7	0.019	-1.45
AKAP6	A-Kinase Anchoring Protein 6	0.021	-1.46
TMEM151A	Transmembrane Protein 151A	0.028	-1.46
GATD3	Glutamine Amidotransferase Class 1 Domain Containing 3	0.019	-1.47
RSPH4A	Radial Spoke Head Component 4A	0.011	-1.47
EVPL	Envoplakin	0.010	-1.47
FEZF2	FEZ Family Zinc Finger 2	0.026	-1.47
MEX3A	Mex-3 RNA Binding Family Member A	0.030	-1.48
RAG1	Recombination Activating 1	0.026	-1.49
SIGLEC12	Sialic Acid Binding Ig Like Lectin 12	0.040	-1.49
SHC3	SHC Adaptor Protein 3	0.040	-1.49
TMC2	Transmembrane Channel Like 2	0.016	-1.51
RHBDF1	Rhomboid 5 Homolog 1	0.047	-1.52

LPIN3	Lipin 3	0.027	-1.52
SPOCK3	SPARC (Osteonectin), Cwcv And Kazal Like Domains Proteoglycan 3	0.032	-1.53
RPL34	Ribosomal Protein L34	0.029	-1.54
BICC1	Bicc Family RNA Binding Protein 1	0.031	-1.54
TGFBR3L	Transforming Growth Factor Beta Receptor 3 Like	0.043	-1.55
STAC	SH3 And Cysteine Rich Domain	0.047	-1.55
KLC3	Kinesin Light Chain 3	0.002	-1.55
BCAM	Basal Cell Adhesion Molecule (Lutheran Blood Group)	0.011	-1.57
AOC1	Amine Oxidase Copper Containing 1	0.042	-1.58
CNNM1	Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 1	0.023	-1.58
MROH7	Maestro Heat Like Repeat Family Member 7	0.017	-1.60
DRD3	Dopamine Receptor D3	0.025	-1.61
FSIP2	Fibrous Sheath Interacting Protein 2	0.039	-1.61
GSTM5	Glutathione S-Transferase Mu 5	0.027	-1.62
LPL	Lipoprotein Lipase	0.005	-1.63
RPL36A	Ribosomal Protein L36a	0.037	-1.65
SAXO2	Stabilizer Of Axonemal Microtubules 2	0.050	-1.66
TAC3	Tachykinin Precursor 3	0.030	-1.66
PNMA8B	PNMA Family Member 8B	0.018	-1.66
PLG	Plasminogen	0.020	-1.68
EFCAB1	EF-Hand Calcium Binding Domain 1	0.040	-1.68
DPP10	Dipeptidyl Peptidase Like 10	0.031	-1.70
VANGL2	VANGL Planar Cell Polarity Protein 2	0.007	-1.73
KCNA1	Potassium Voltage-Gated Channel Subfamily A Member 1	0.009	-1.74
FOLR3	Folate Receptor Gamma	0.002	-1.75
NKAIN2	Sodium/Potassium Transporting Atpase Interacting 2	0.008	-1.77
HOXA7	Homeobox A7	0.009	-1.79
SLC30A3	Solute Carrier Family 30 Member 3	0.009	-1.79

RPRML	Reprimo Like	0.007	-1.84
TNS2	Tensin 2	< 0.001	-1.85
MANSC4	MANSC Domain Containing 4	0.011	-1.86
PCDHGA5	Protocadherin Gamma Subfamily A, 5	0.041	-1.87
GRIP1	Glutamate Receptor Interacting Protein 1	0.001	-1.88
SYN3	Synapsin III	0.016	-1.89
AANAT	Aralkylamine N-Acetyltransferase	0.037	-1.95
MAGED4	MAGE Family Member D4	0.028	-1.97
VPREB1	V-Set Pre-B Cell Surrogate Light Chain 1	0.022	-1.97
KCNJ8	Potassium Inwardly Rectifying Channel Subfamily J Member 8	0.046	-2.00
LEKR1	Leucine, Glutamate And Lysine Rich 1	0.005	-2.02
CHST6	Carbohydrate Sulfotransferase 6	0.007	-2.04
TACSTD2	Tumor Associated Calcium Signal Transducer 2	< 0.001	-2.10
MSLN	Mesothelin	0.005	-2.26
TNNI3	Troponin I3, Cardiac Type	0.012	-2.29
NAALAD2	N-Acetylated Alpha-Linked Acidic Dipeptidase 2	0.006	-2.33
LPAR4	Lysophosphatidic Acid Receptor 4	0.011	-2.33
PCDHA4	Protocadherin Alpha 4	0.038	-2.34
CBLN2	Cerebellin 2 Precursor	0.002	-2.45
POU2F3	POU Class 2 Homeobox 3	0.007	-2.59
PNMA8A	PNMA Family Member 8A	0.017	-2.66
NXF3	Nuclear RNA Export Factor 3	0.015	-2.74
MTRNR2L8	MT-RNR2 Like 8	< 0.001	-2.75
GRIP2	Glutamate Receptor Interacting Protein 2	0.002	-2.76
TACR3	Tachykinin Receptor 3	0.038	-2.77
C4BPA	Complement Component 4 Binding Protein Alpha	< 0.001	-2.86
RNF150	Ring Finger Protein 150	0.001	-3.32

Table 2E. List of 461 genes differently modulated in MDD vs LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05).

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3	TBC1 Domain Family Member 3	0.001	13.97
OR6N1	Olfactory Receptor Family 6 Subfamily N Member 1	0.007	2.77
PRSS50	Serine Protease 50	0.007	2.64
DCN	Decorin	0.002	2.36
C2CD6	C2 Calcium Dependent Domain Containing 6	0.039	2.33
ITGA8	Integrin Subunit Alpha 8	0.013	2.31
SRGAP1	SLIT-ROBO Rho Gtpase Activating Protein 1	< 0.001	2.16
FBXO16	F-Box Protein 16	0.014	2.16
INTU	Inturned Planar Cell Polarity Protein	0.012	2.07
RGS13	Regulator Of G Protein Signaling 13	0.014	1.99
TULP2	TUB Like Protein 2	0.044	1.96
DNAH17	Dynein Axonemal Heavy Chain 17	0.001	1.96
IFI44L	Interferon Induced Protein 44 Like	0.005	1.93
FTCD	Formimidoyltransferase Cyclodeaminase	0.027	1.92
C9orf152	Chromosome 9 Open Reading Frame 152	0.024	1.89
KLF14	Kruppel Like Factor 14	0.039	1.88
DZIP1L	DAZ Interacting Zinc Finger Protein 1 Like	< 0.001	1.87
RSAD2	Radical S-Adenosyl Methionine Domain Containing 2	0.016	1.87
SIGLEC1	Sialic Acid Binding Ig Like Lectin 1	0.005	1.86
CCL2	C-C Motif Chemokine Ligand 2	0.016	1.85
SCUBE2	Signal Peptide, CUB Domain And EGF Like Domain Containing 2	0.007	1.83
INAVA	Innate Immunity Activator	0.002	1.83
APOBEC3B	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3B	0.003	1.82
RNASE1	Ribonuclease A Family Member 1, Pancreatic	0.002	1.80
VEGFC	Vascular Endothelial Growth Factor C	0.003	1.79
OLFM4	Olfactomedin 4	0.009	1.76

TSPO2	Translocator Protein 2	0.001	1.74
ABCC11	ATP Binding Cassette Subfamily C Member 11	0.030	1.73
CMPK2	Cytidine/Uridine Monophosphate Kinase 2	0.011	1.69
PPARG	Peroxisome Proliferator Activated Receptor Gamma	0.010	1.68
IFIT1	Interferon Induced Protein With Tetratricopeptide Repeats 1	0.019	1.68
NEK5	NIMA Related Kinase 5	0.046	1.68
SPATA1	Spermatogenesis Associated 1	0.001	1.67
IFI44	Interferon Induced Protein 44	0.013	1.66
KNL1	Kinetochore Scaffold 1	0.002	1.66
LOC112267968	Uncharacterized LOC112267968	0.017	1.63
RNASE2	Ribonuclease A Family Member 2	0.001	1.63
ISG15	ISG15 Ubiquitin Like Modifier	0.020	1.63
BMP8A	Bone Morphogenetic Protein 8a	0.007	1.63
HTRA3	Htra Serine Peptidase 3	0.008	1.62
USP18	Ubiquitin Specific Peptidase 18	0.008	1.62
PLEKHH2	Pleckstrin Homology, Myth4 And FERM Domain Containing H2	0.027	1.62
PKP3	Plakophilin 3	0.038	1.62
FPGT-TNNI3K	FPGT-TNNI3K Readthrough	0.035	1.61
NECTIN2	Nectin Cell Adhesion Molecule 2	0.019	1.59
KCNMA1	Potassium Calcium-Activated Channel Subfamily M Alpha 1	0.009	1.59
CEACAM8	CEA Cell Adhesion Molecule 8	0.014	1.58
MMP8	Matrix Metallopeptidase 8	0.011	1.58
RBM24	RNA Binding Motif Protein 24	0.043	1.58
PAQR5	Progestin And Adipoq Receptor Family Member 5	0.019	1.58
LTF	Lactotransferrin	0.009	1.57
RFX8	Regulatory Factor X8	0.023	1.56
TMEM119	Transmembrane Protein 119	0.005	1.56
FGF17	Fibroblast Growth Factor 17	0.040	1.55

OAS3	2'-5'-Oligoadenylate Synthetase 3	0.030	1.55
IFIT3	Interferon Induced Protein With Tetratricopeptide Repeats 3	0.017	1.54
SAMD13	Sterile Alpha Motif Domain Containing 13	0.038	1.54
KIF7	Kinesin Family Member 7	0.003	1.54
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.019	1.53
DEFA4	Defensin Alpha 4	0.041	1.52
C1QB	Complement C1q B Chain	0.020	1.51
TNFAIP6	TNF Alpha Induced Protein 6	0.007	1.50
SDC1	Syndecan 1	0.046	1.50
MIXL1	Mix Paired-Like Homeobox	0.030	1.50
RSPH9	Radial Spoke Head Component 9	0.004	1.49
ALPK3	Alpha Kinase 3	0.001	1.49
ZPBP2	Zona Pellucida Binding Protein 2	0.046	1.48
MX1	MX Dynamin Like Gtpase 1	0.024	1.48
C1QC	Complement C1q C Chain	0.045	1.48
IFI6	Interferon Alpha Inducible Protein 6	0.030	1.48
KIF4A	Kinesin Family Member 4A	0.015	1.47
SPATS2L	Spermatogenesis Associated Serine Rich 2 Like	0.016	1.47
LAMP3	Lysosomal Associated Membrane Protein 3	0.036	1.47
SEMG1	Semenogelin 1	0.009	1.47
RNF43	Ring Finger Protein 43	0.013	1.47
IFIT2	Interferon Induced Protein With Tetratricopeptide Repeats 2	0.010	1.46
ADGRG6	Adhesion G Protein-Coupled Receptor G6	0.043	1.46
SMIM10	Small Integral Membrane Protein 10	0.018	1.46
ELANE	Elastase, Neutrophil Expressed	0.016	1.45
ARHGAP23	Rho Gtpase Activating Protein 23	0.008	1.45
HERC6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6	0.001	1.45
SDS	Serine Dehydratase	0.015	1.45
GRIN3B	Glutamate Ionotropic Receptor NMDA Type Subunit 3B	0.013	1.44

TRIM6	Tripartite Motif Containing 6	0.041	1.44
BRCA2	BRCA2 DNA Repair Associated	0.033	1.43
CRISP3	Cysteine Rich Secretory Protein 3	0.006	1.43
OSCP1	Organic Solute Carrier Partner 1	0.014	1.43
MYH11	Myosin Heavy Chain 11	0.033	1.43
ABCG2	ATP Binding Cassette Subfamily G Member 2 (Junior Blood Group)	0.024	1.42
LRRIQ3	Leucine Rich Repeats And IQ Motif Containing 3	0.048	1.42
GLDC	Glycine Decarboxylase	0.045	1.42
LGALS4	Galectin 4	0.019	1.42
E2F7	E2F Transcription Factor 7	0.041	1.42
RHD	Rh Blood Group D Antigen	0.045	1.42
AXL	AXL Receptor Tyrosine Kinase	0.016	1.40
LRRC70	Leucine Rich Repeat Containing 70	0.024	1.39
CD80	CD80 Molecule	0.014	1.39
POLN	DNA Polymerase Nu	0.017	1.39
HPN	Hepsin	0.045	1.38
ASPM	Assembly Factor For Spindle Microtubules	0.021	1.38
BUB1B	BUB1 Mitotic Checkpoint Serine/Threonine Kinase B	0.016	1.38
ABCA13	ATP Binding Cassette Subfamily A Member 13	0.040	1.38
TMEM255B	Transmembrane Protein 255B	0.006	1.38
MGAM2	Maltase-Glucoamylase 2 (Putative)	0.012	1.38
LAMA2	Laminin Subunit Alpha 2	0.041	1.38
CFAP54	Cilia And Flagella Associated Protein 54	0.042	1.37
TP63	Tumor Protein P63	0.048	1.37
LCN2	Lipocalin 2	0.036	1.37
AGRN	Agrin	0.043	1.37
CRHBP	Corticotropin Releasing Hormone Binding Protein	0.019	1.36
BAMBI	BMP And Activin Membrane Bound Inhibitor	0.033	1.36

CAV2	Caveolin 2	0.047	1.36
FFAR3	Free Fatty Acid Receptor 3	0.039	1.35
OAS2	2'-5'-Oligoadenylate Synthetase 2	0.029	1.35
ITGA9	Integrin Subunit Alpha 9	0.032	1.35
HPDL	4-Hydroxyphenylpyruvate Dioxygenase Like	0.042	1.34
EIF2AK2	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2	0.020	1.34
CHDH	Choline Dehydrogenase	0.019	1.34
HORMAD1	HORMA Domain Containing 1	0.044	1.33
NEXN	Nexilin F-Actin Binding Protein	0.038	1.33
MZB1	Marginal Zone B And B1 Cell Specific Protein	0.039	1.33
RASSF6	Ras Association Domain Family Member 6	0.036	1.33
XAF1	XIAP Associated Factor 1	0.043	1.33
IFIH1	Interferon Induced With Helicase C Domain 1	0.005	1.33
PTH2R	Parathyroid Hormone 2 Receptor	0.044	1.33
ADAMDEC 1	ADAM Like Decysin 1	0.037	1.33
PLSCR1	Phospholipid Scramblase 1	0.023	1.32
CHIT1	Chitinase 1	0.024	1.32
FAM174B	Family With Sequence Similarity 174 Member B	0.002	1.32
CASP5	Caspase 5	0.040	1.31
SCN4B	Sodium Voltage-Gated Channel Beta Subunit 4	0.048	1.31
XCL1	X-C Motif Chemokine Ligand 1	0.017	1.31
CACNA1E	Calcium Voltage-Gated Channel Subunit Alpha1 E	0.022	1.31
SHISA8	Shisa Family Member 8	0.028	1.30
DDX60	Dexd/H-Box Helicase 60	0.040	1.30
EDA	Ectodysplasin A	0.044	1.30
PRG4	Proteoglycan 4	0.049	1.29
AIM2	Absent In Melanoma 2	0.030	1.29
TXNDC5	Thioredoxin Domain Containing 5	0.049	1.29

LOC400499	Putative Uncharacterized Protein LOC400499	0.003	1.29
DDX60L	Dexd/H-Box 60 Like	0.015	1.29
FRMD3	FERM Domain Containing 3	0.015	1.29
RIBC1	RIB43A Domain With Coiled-Coils 1	0.015	1.29
DCAF4L1	DDB1 And CUL4 Associated Factor 4 Like 1	0.009	1.28
COCH	Cochlin	0.022	1.28
MT2A	Metallothionein 2A	0.039	1.28
IFIT5	Interferon Induced Protein With Tetratricopeptide Repeats 5	0.046	1.28
SFN	Stratifin	0.038	1.28
TMC4	Transmembrane Channel Like 4	0.046	1.28
DOCK4	Dedicator Of Cytokinesis 4	0.014	1.28
GABRR2	Gamma-Aminobutyric Acid Type A Receptor Subunit Rho2	0.023	1.28
XRRA1	X-Ray Radiation Resistance Associated 1	0.035	1.27
ZNF714	Zinc Finger Protein 714	0.017	1.27
LGALSL	Galectin Like	0.017	1.26
RAB23	RAB23, Member RAS Oncogene Family	0.029	1.26
SAMD9L	Sterile Alpha Motif Domain Containing 9 Like	0.035	1.25
AFDN	Afadin, Adherens Junction Formation Factor	0.036	1.25
ATP8B4	Atpase Phospholipid Transporting 8B4 (Putative)	0.001	1.25
ADM	Adrenomedullin	0.025	1.25
DDX58	Dexd/H-Box Helicase 58	0.040	1.25
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.018	1.24
B4GALT6	Beta-1,4-Galactosyltransferase 6	0.024	1.24
CFAP45	Cilia And Flagella Associated Protein 45	0.014	1.24
ODF3B	Outer Dense Fiber Of Sperm Tails 3B	0.030	1.24
IRF7	Interferon Regulatory Factor 7	0.046	1.24
PARP9	Poly(ADP-Ribose) Polymerase Family Member 9	0.028	1.24
TRIM22	Tripartite Motif Containing 22	0.031	1.24
SAMD9	Sterile Alpha Motif Domain Containing 9	0.016	1.24

ZBP1	Z-DNA Binding Protein 1	0.039	1.24
KIAA0319	Kiaa0319	0.006	1.24
CRABP2	Cellular Retinoic Acid Binding Protein 2	0.017	1.23
HCAR3	Hydroxycarboxylic Acid Receptor 3	0.007	1.23
NUGGC	Nuclear Gtpase, Germinal Center Associated	0.006	1.23
AGBL2	AGBL Carboxypeptidase 2	0.027	1.23
STEAP4	STEAP4 Metalloreductase	0.014	1.23
PTPN13	Protein Tyrosine Phosphatase Non-Receptor Type 13	0.030	1.23
TNFSF10	TNF Superfamily Member 10	0.045	1.23
NT5C3A	5'-Nucleotidase, Cytosolic IIIA	0.016	1.22
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3A	0.012	1.22
TCN2	Transcobalamin 2	0.044	1.22
KIAA1958	Kiaa1958	0.033	1.22
ELL2	Elongation Factor For RNA Polymerase II 2	0.004	1.22
C5orf34	Chromosome 5 Open Reading Frame 34	0.041	1.22
NSUN7	NOP2/Sun RNA Methyltransferase Family Member 7	0.023	1.22
MX2	MX Dynamin Like Gtpase 2	0.017	1.22
BMP6	Bone Morphogenetic Protein 6	0.004	1.22
SCO2	Synthesis Of Cytochrome C Oxidase 2	0.033	1.22
HELZ2	Helicase With Zinc Finger 2	0.048	1.21
NOXRED1	NADP Dependent Oxidoreductase Domain Containing 1	0.048	1.21
KCNJ2	Potassium Inwardly Rectifying Channel Subfamily J Member 2	0.011	1.21
ZNF107	Zinc Finger Protein 107	0.037	1.21
GGH	Gamma-Glutamyl Hydrolase	0.039	1.21
HCAR2	Hydroxycarboxylic Acid Receptor 2	0.011	1.21
SLFN12	Schlafen Family Member 12	0.015	1.21
TUT1	Terminal Uridylyl Transferase 1, U6 Snrna-Specific	0.021	1.21
KIF24	Kinesin Family Member 24	0.041	1.21
NHS	NHS Actin Remodeling Regulator	0.027	1.21
SMAD1	SMAD Family Member 1	0.041	1.21

KIAA0825	Kiaa0825	0.023	1.21
SGPP2	Sphingosine-1-Phosphate Phosphatase 2	0.004	1.21
PRRG4	Proline Rich And Gla Domain 4	0.019	1.21
MAP3K7CL	MAP3K7 C-Terminal Like	0.012	1.20
TLR5	Toll Like Receptor 5	0.035	1.20
C18orf54	Chromosome 18 Open Reading Frame 54	0.029	1.20
IL1RN	Interleukin 1 Receptor Antagonist	0.027	1.20
NTNG2	Netrin G2	0.040	1.20
SV2A	Synaptic Vesicle Glycoprotein 2A	0.014	-1.20
PRICKLE1	Prickle Planar Cell Polarity Protein 1	0.007	-1.20
BBS7	Bardet-Biedl Syndrome 7	0.019	-1.20
NLGN2	Neuroligin 2	0.034	-1.20
WASF1	WASP Family Member 1	0.018	-1.21
CBR3	Carbonyl Reductase 3	0.026	-1.21
SLC9A3R2	SLC9A3 Regulator 2	0.004	-1.21
ARMCX2	Armadillo Repeat Containing X-Linked 2	0.024	-1.21
IGSF9B	Immunoglobulin Superfamily Member 9B	0.050	-1.21
KIF9	Kinesin Family Member 9	0.049	-1.21
SYP	Synaptophysin	0.043	-1.22
PRRT1	Proline Rich Transmembrane Protein 1	0.013	-1.22
ENC1	Ectodermal-Neural Cortex 1	0.011	-1.22
BFSP1	Beaded Filament Structural Protein 1	0.041	-1.22
CD248	CD248 Molecule	0.005	-1.22
CRB3	Crumbs Cell Polarity Complex Component 3	0.026	-1.22
ATP1B1	Atpase Na+/K+ Transporting Subunit Beta 1	0.024	-1.23
GSTA4	Glutathione S-Transferase Alpha 4	0.023	-1.23
SPAG8	Sperm Associated Antigen 8	0.015	-1.23
PFN2	Profilin 2	0.039	-1.23
MAD1L1	Mitotic Arrest Deficient 1 Like 1	0.002	-1.23
NET1	Neuroepithelial Cell Transforming 1	0.001	-1.23

MTMR8	Myotubularin Related Protein 8	0.044	-1.23
TESC	Tescalcin	0.014	-1.23
ZRANB3	Zinc Finger RANBP2-Type Containing 3	0.045	-1.24
PLPP3	Phospholipid Phosphatase 3	0.024	-1.24
FBLN2	Fibulin 2	0.005	-1.24
PSD	Pleckstrin And Sec7 Domain Containing	0.013	-1.25
ARHGAP39	Rho Gtpase Activating Protein 39	0.047	-1.25
SLC5A10	Solute Carrier Family 5 Member 10	0.037	-1.25
SUSD4	Sushi Domain Containing 4	0.028	-1.25
FAAH	Fatty Acid Amide Hydrolase	0.001	-1.26
FILIP1L	Filamin A Interacting Protein 1 Like	0.015	-1.26
BSN	Bassoon Presynaptic Cytomatrix Protein	0.030	-1.26
KLHL33	Kelch Like Family Member 33	0.031	-1.26
ZNF667	Zinc Finger Protein 667	0.043	-1.26
NR4A1	Nuclear Receptor Subfamily 4 Group A Member 1	0.007	-1.26
SMARCA1	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 1	0.028	-1.26
SLC22A17	Solute Carrier Family 22 Member 17	0.012	-1.26
ZFP2	ZFP2 Zinc Finger Protein	0.041	-1.26
DLGAP3	DLG Associated Protein 3	0.028	-1.26
RTN4RL2	Reticulon 4 Receptor Like 2	0.042	-1.26
STRBP	Spermatid Perinuclear RNA Binding Protein	< 0.001	-1.26
EEF1A2	Eukaryotic Translation Elongation Factor 1 Alpha 2	0.034	-1.27
ZNF563	Zinc Finger Protein 563	0.031	-1.27
TSPAN7	Tetraspanin 7	0.047	-1.27
EIF5AL1	Eukaryotic Translation Initiation Factor 5A Like 1	0.041	-1.27
C1QTNF4	C1q And TNF Related 4	0.045	-1.27
EFNB2	Ephrin B2	0.014	-1.27
SERPINE2	Serpin Family E Member 2	0.007	-1.27
ADGRA3	Adhesion G Protein-Coupled Receptor A3	0.004	-1.27

STXBP1	Syntaxin Binding Protein 1	0.007	-1.28
DBNDD1	Dysbindin Domain Containing 1	0.010	-1.28
SYBU	Syntabulin	0.020	-1.28
MACROD2	Mono-ADP Ribosylhydrolase 2	0.012	-1.28
MAPT	Microtubule Associated Protein Tau	0.036	-1.28
MOCS1	Molybdenum Cofactor Synthesis 1	0.016	-1.28
SARDH	Sarcosine Dehydrogenase	0.004	-1.28
ELFN2	Extracellular Leucine Rich Repeat And Fibronectin Type III Domain Containing 2	0.006	-1.28
PDZK1IP1	PDZK1 Interacting Protein 1	0.034	-1.29
SLC7A8	Solute Carrier Family 7 Member 8	0.027	-1.29
PTPRF	Protein Tyrosine Phosphatase Receptor Type F	0.036	-1.29
TXNRD3	Thioredoxin Reductase 3	0.005	-1.29
DLGAP1	DLG Associated Protein 1	0.044	-1.29
SOX8	SRY-Box Transcription Factor 8	0.005	-1.29
PGLYRP2	Peptidoglycan Recognition Protein 2	0.036	-1.29
SLC17A7	Solute Carrier Family 17 Member 7	0.037	-1.29
P3H2	Prolyl 3-Hydroxylase 2	0.033	-1.30
TUSC3	Tumor Suppressor Candidate 3	0.045	-1.30
DIRAS1	DIRAS Family Gtpase 1	0.027	-1.30
FAIM2	Fas Apoptotic Inhibitory Molecule 2	0.046	-1.30
MFSD6L	Major Facilitator Superfamily Domain Containing 6 Like	0.043	-1.30
FBXL16	F-Box And Leucine Rich Repeat Protein 16	0.011	-1.31
CNN3	Calponin 3	0.002	-1.31
CAMK2A	Calcium/Calmodulin Dependent Protein Kinase II Alpha	0.028	-1.31
TFCP2L1	Transcription Factor CP2 Like 1	0.033	-1.32
RASL10B	RAS Like Family 10 Member B	0.027	-1.32
GRIA2	Glutamate Ionotropic Receptor AMPA Type Subunit 2	0.036	-1.32
MAP2	Microtubule Associated Protein 2	0.020	-1.32
CELF3	CUGBP Elav-Like Family Member 3	0.042	-1.32

ANK2	Ankyrin 2	0.033	-1.32
FGFR4	Fibroblast Growth Factor Receptor 4	0.021	-1.32
DPF3	Double PHD Fingers 3	0.004	-1.33
KHDRBS3	KH RNA Binding Domain Containing, Signal Transduction Associated 3	0.042	-1.33
GAD1	Glutamate Decarboxylase 1	0.049	-1.33
CDK5R2	Cyclin Dependent Kinase 5 Regulatory Subunit 2	0.028	-1.33
SLC1A2	Solute Carrier Family 1 Member 2	0.048	-1.34
NDRG4	NDRG Family Member 4	0.024	-1.34
GCAT	Glycine C-Acetyltransferase	0.009	-1.34
TAGLN3	Transgelin 3	0.025	-1.34
NPTX1	Neuronal Pentraxin 1	0.007	-1.34
PRH1	Proline Rich Protein Haeiii Subfamily 1	0.012	-1.34
CSPG5	Chondroitin Sulfate Proteoglycan 5	0.042	-1.35
DSCAML1	DS Cell Adhesion Molecule Like 1	0.032	-1.35
PDE8B	Phosphodiesterase 8B	0.025	-1.35
NRXN2	Neurexin 2	0.024	-1.35
GYPE	Glycophorin E (MNS Blood Group)	0.049	-1.35
ACTG2	Actin Gamma 2, Smooth Muscle	0.030	-1.36
CC2D2A	Coiled-Coil And C2 Domain Containing 2A	0.017	-1.36
HOMER1	Homer Scaffold Protein 1	0.014	-1.37
GPM6A	Glycoprotein M6A	0.036	-1.37
ANKRD31	Ankyrin Repeat Domain 31	0.034	-1.37
PDZD7	PDZ Domain Containing 7	0.038	-1.37
RAPGEF5	Rap Guanine Nucleotide Exchange Factor 5	0.039	-1.37
LRP5	LDL Receptor Related Protein 5	0.005	-1.38
RYR1	Ryanodine Receptor 1	0.012	-1.38
GPC3	Glypican 3	0.015	-1.38
GRIA1	Glutamate Ionotropic Receptor AMPA Type Subunit 1	0.047	-1.38
NALF1	NALCN Channel Auxiliary Factor 1	0.047	-1.38

STXBP4	Syntaxin Binding Protein 4	0.001	-1.39
PFDN4	Prefoldin Subunit 4	0.034	-1.39
KLC3	Kinesin Light Chain 3	0.011	-1.39
ZNF835	Zinc Finger Protein 835	0.005	-1.39
TNNT1	Troponin T1, Slow Skeletal Type	0.040	-1.39
DEGS2	Delta 4-Desaturase, Sphingolipid 2	0.004	-1.40
PPP2R2C	Protein Phosphatase 2 Regulatory Subunit Bgamma	0.026	-1.40
SPRED3	Sprouty Related EVH1 Domain Containing 3	0.015	-1.40
SOBP	Sine Oculis Binding Protein Homolog	0.006	-1.40
KRT2	Keratin 2	0.023	-1.40
HPCA	Hippocalcin	0.012	-1.40
POTEI	POTE Ankyrin Domain Family Member I	0.017	-1.40
SOX2	SRY-Box Transcription Factor 2	0.042	-1.40
SLC16A11	Solute Carrier Family 16 Member 11	< 0.001	-1.41
ATP2B2	Atpase Plasma Membrane Ca2+ Transporting 2	0.046	-1.41
CCK	Cholecystokinin	0.015	-1.42
KCNC1	Potassium Voltage-Gated Channel Subfamily C Member 1	0.034	-1.42
SDC2	Syndecan 2	0.042	-1.42
COLEC12	Collectin Subfamily Member 12	0.030	-1.42
TNR	Tenascin R	0.041	-1.42
GALNT16	Polypeptide N-Acetylgalactosaminyltransferase 16	0.040	-1.43
TTC9B	Tetratricopeptide Repeat Domain 9B	0.038	-1.43
PRSS57	Serine Protease 57	0.004	-1.43
MMEL1	Membrane Metalloendopeptidase Like 1	0.004	-1.43
ATP1A3	Atpase Na+/K+ Transporting Subunit Alpha 3	0.003	-1.43
POTEM	POTE Ankyrin Domain Family Member M	0.015	-1.44
CAMSAP3	Calmodulin Regulated Spectrin Associated Protein Family Member 3	0.017	-1.44
TEDC2	Tubulin Epsilon And Delta Complex 2	0.022	-1.44
TTYH1	Tweety Family Member 1	0.018	-1.44

KIF5A	Kinesin Family Member 5A	0.010	-1.44
EPB41L1	Erythrocyte Membrane Protein Band 4.1 Like 1	0.013	-1.45
STMN2	Stathmin 2	0.013	-1.45
MXRA8	Matrix Remodeling Associated 8	0.004	-1.46
TUBB3	Tubulin Beta 3 Class III	0.012	-1.46
APBA1	Amyloid Beta Precursor Protein Binding Family A Member 1	0.002	-1.47
ACTC1	Actin Alpha Cardiac Muscle 1	0.046	-1.47
EPHA7	EPH Receptor A7	0.023	-1.47
RGPD3	RANBP2 Like And GRIP Domain Containing 3	0.016	-1.48
SLCO1C1	Solute Carrier Organic Anion Transporter Family Member 1C1	0.035	-1.48
GRIA3	Glutamate Ionotropic Receptor AMPA Type Subunit 3	0.027	-1.48
FHAD1	Forkhead Associated Phosphopeptide Binding Domain 1	0.029	-1.48
BRINP1	BMP/Retinoic Acid Inducible Neural Specific 1	0.004	-1.49
SEZ6L2	Seizure Related 6 Homolog Like 2	0.006	-1.49
GRIP1	Glutamate Receptor Interacting Protein 1	0.040	-1.50
EPHA5	EPH Receptor A5	0.039	-1.50
DPYSL4	Dihydropyrimidinase Like 4	0.048	-1.50
ABCA6	ATP Binding Cassette Subfamily A Member 6	0.007	-1.51
FHOD3	Formin Homology 2 Domain Containing 3	0.027	-1.51
PPM1E	Protein Phosphatase, Mg2+/Mn2+ Dependent 1E	0.030	-1.51
PROM2	Prominin 2	0.018	-1.51
RBFOX2	RNA Binding Fox-1 Homolog 2	0.003	-1.51
MYO1A	Myosin IA	0.003	-1.52
GRM7	Glutamate Metabotropic Receptor 7	0.007	-1.53
BCAM	Basal Cell Adhesion Molecule (Lutheran Blood Group)	0.015	-1.53
STARD13	Star Related Lipid Transfer Domain Containing 13	0.003	-1.53
PTPN20	Protein Tyrosine Phosphatase Non-Receptor Type 20	0.035	-1.54
KNDC1	Kinase Non-Catalytic C-Lobe Domain Containing 1	0.001	-1.54
DCLK1	Doublecortin Like Kinase 1	0.006	-1.55
ADCY2	Adenylate Cyclase 2	0.020	-1.55

IGSF21	Immunoglobin Superfamily Member 21	0.039	-1.56
BRINP2	BMP/Retinoic Acid Inducible Neural Specific 2	0.008	-1.56
MYH10	Myosin Heavy Chain 10	0.004	-1.56
STAC2	SH3 And Cysteine Rich Domain 2	0.002	-1.57
EVPL	Envoplakin	0.001	-1.58
RGS4	Regulator Of G Protein Signaling 4	0.030	-1.58
GRIN1	Glutamate Ionotropic Receptor NMDA Type Subunit 1	0.007	-1.58
AOC1	Amine Oxidase Copper Containing 1	0.049	-1.59
RASD2	RASD Family Member 2	0.031	-1.59
MAGI1	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 1	0.030	-1.59
SULT1A4	Sulfotransferase Family 1A Member 4	0.037	-1.59
DLL3	Delta Like Canonical Notch Ligand 3	0.021	-1.59
TUBB8B	Tubulin Beta 8B	0.042	-1.60
NKAIN2	Sodium/Potassium Transporting Atpase Interacting 2	0.006	-1.60
ADGRL3	Adhesion G Protein-Coupled Receptor L3	0.007	-1.61
GABRA3	Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha3	0.010	-1.61
NRCAM	Neuronal Cell Adhesion Molecule	< 0.001	-1.62
LRRC36	Leucine Rich Repeat Containing 36	0.001	-1.63
MAPK4	Mitogen-Activated Protein Kinase 4	0.047	-1.63
CHRNB2	Cholinergic Receptor Nicotinic Beta 2 Subunit	0.046	-1.63
RAG1	Recombination Activating 1	0.008	-1.63
LMOD1	Leiomodin 1	0.013	-1.64
CTTNBP2	Cortactin Binding Protein 2	0.002	-1.64
BHLHE22	Basic Helix-Loop-Helix Family Member E22	0.014	-1.65
LPL	Lipoprotein Lipase	0.004	-1.66
MEIOC	Meiosis Specific With Coiled-Coil Domain	0.048	-1.66
HOXA5	Homeobox A5	0.026	-1.67
TGFBR3L	Transforming Growth Factor Beta Receptor 3 Like	0.018	-1.67
STXBP6	Syntaxin Binding Protein 6	0.013	-1.67

LCA5	Lebercilin LCA5	0.006	-1.69
RIMS4	Regulating Synaptic Membrane Exocytosis 4	0.047	-1.69
VSTM2B	V-Set And Transmembrane Domain Containing 2B	0.016	-1.70
FEZF2	FEZ Family Zinc Finger 2	0.002	-1.70
TMEM132A	Transmembrane Protein 132A	0.002	-1.70
FOXH1	Forkhead Box H1	0.017	-1.71
ASIC2	Acid Sensing Ion Channel Subunit 2	0.006	-1.71
NTRK3	Neurotrophic Receptor Tyrosine Kinase 3	0.006	-1.71
FNDC5	Fibronectin Type III Domain Containing 5	0.008	-1.72
ERBB4	Erb-B2 Receptor Tyrosine Kinase 4	0.010	-1.73
KCNAB1	Potassium Voltage-Gated Channel Subfamily A Regulatory Beta Subunit 1	0.004	-1.73
GPR176	G Protein-Coupled Receptor 176	0.017	-1.73
TTLL10	Tubulin Tyrosine Ligase Like 10	0.001	-1.74
TJP1	Tight Junction Protein 1	0.002	-1.77
GLRB	Glycine Receptor Beta	0.012	-1.78
TACSTD2	Tumor Associated Calcium Signal Transducer 2	0.005	-1.79
SYN3	Synapsin III	0.026	-1.80
SHC3	SHC Adaptor Protein 3	0.009	-1.80
GABRD	Gamma-Aminobutyric Acid Type A Receptor Subunit Delta	0.004	-1.82
DMRTC1	DMRT Like Family C1	0.028	-1.83
ABCC8	ATP Binding Cassette Subfamily C Member 8	0.023	-1.87
FIBCD1	Fibrinogen C Domain Containing 1	0.016	-1.87
GSTA1	Glutathione S-Transferase Alpha 1	0.009	-1.87
MESP2	Mesoderm Posterior Bhlh Transcription Factor 2	0.016	-1.88
GLRA2	Glycine Receptor Alpha 2	0.027	-1.89
PLS3	Plastin 3	< 0.001	-1.90
RALYL	RALY RNA Binding Protein Like	0.001	-1.94
RGS11	Regulator Of G Protein Signaling 11	0.047	-1.96

INSYN2A	Inhibitory Synaptic Factor 2A	0.008	-1.96
FOLR3	Folate Receptor Gamma	< 0.001	-1.99
KCNJ10	Potassium Inwardly Rectifying Channel Subfamily J Member 10	0.001	-1.99
DNAH11	Dynein Axonemal Heavy Chain 11	0.016	-1.99
HTR2B	5-Hydroxytryptamine Receptor 2B	0.033	-1.99
CASQ1	Calsequestrin 1	0.029	-2.08
DNAAF3	Dynein Axonemal Assembly Factor 3	0.038	-2.10
MAGI2	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2	0.003	-2.18
CFAP61	Cilia And Flagella Associated Protein 61	0.029	-2.19
KCNQ2	Potassium Voltage-Gated Channel Subfamily Q Member 2	0.003	-2.20
GRIP2	Glutamate Receptor Interacting Protein 2	0.022	-2.23
HOXA7	Homeobox A7	< 0.001	-2.26
SLC12A1	Solute Carrier Family 12 Member 1	0.014	-2.28
TP53TG3D	TP53 Target 3D	0.029	-2.29
MEDAG	Mesenteric Estrogen Dependent Adipogenesis	0.003	-2.33
VWA3B	Von Willebrand Factor A Domain Containing 3B	0.028	-2.35
RPS4Y2	Ribosomal Protein S4 Y-Linked 2	0.046	-2.37
GRM6	Glutamate Metabotropic Receptor 6	0.033	-2.39
NPR1	Natriuretic Peptide Receptor 1	0.016	-2.48
CFAP46	Cilia And Flagella Associated Protein 46	0.002	-2.55
TACR3	Tachykinin Receptor 3	0.027	-2.59
TREML4	Triggering Receptor Expressed On Myeloid Cells Like 4	0.024	-2.61
CRHR1	Corticotropin Releasing Hormone Receptor 1	0.020	-2.71
NAALAD2	N-Acetylated Alpha-Linked Acidic Dipeptidase 2	0.004	-2.77
TFAP2A	Transcription Factor AP-2 Alpha	0.016	-2.80
TNNI3	Troponin I3, Cardiac Type	0.002	-2.97
CBLN2	Cerebellin 2 Precursor	< 0.001	-3.00
CYP26B1	Cytochrome P450 Family 26 Subfamily B Member 1	0.040	-3.48
C4BPA	Complement Component 4 Binding Protein Alpha	< 0.001	-3.65

TBC1D3H	TBC1 Domain Family Member 3H	< 0.001	-1140.3

Table 3E. List of 192 genes differently modulated in HR vs LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
FBXO10	F-Box Protein 10	0.007	3.62
FBXO16	F-Box Protein 16	0.002	2.62
CNDP1	Carnosine Dipeptidase 1	0.002	2.34
MTRNR2L8	MT-RNR2 Like 8	0.002	2.31
RNF150	Ring Finger Protein 150	0.015	2.28
NECTIN2	Nectin Cell Adhesion Molecule 2	< 0.001	2.22
TPO	Thyroid Peroxidase	0.002	2.17
HSPB6	Heat Shock Protein Family B (Small) Member 6	0.029	2.13
MANSC4	MANSC Domain Containing 4	0.005	2.13
CHST6	Carbohydrate Sulfotransferase 6	0.007	2.09
H3C12	H3 Clustered Histone 12	0.033	2.06
VPREB1	V-Set Pre-B Cell Surrogate Light Chain 1	0.014	2.01
TWIST2	Twist Family Bhlh Transcription Factor 2	0.015	1.93
USP50	Ubiquitin Specific Peptidase 50	0.026	1.91
SRGAP1	SLIT-ROBO Rho Gtpase Activating Protein 1	0.001	1.86
LEKR1	Leucine, Glutamate And Lysine Rich 1	0.011	1.84
RYR2	Ryanodine Receptor 2	0.011	1.84
RHOD	Ras Homolog Family Member D	0.010	1.83
PLG	Plasminogen	0.023	1.73
MYOM2	Myomesin 2	0.049	1.71
COL16A1	Collagen Type XVI Alpha 1 Chain	0.032	1.68
DNAI3	Dynein Axonemal Intermediate Chain 3	0.029	1.65
SLC2A10	Solute Carrier Family 2 Member 10	0.040	1.65
HHIPL1	HHIP Like 1	0.046	1.62
SLC25A27	Solute Carrier Family 25 Member 27	0.028	1.62
FOXD1	Forkhead Box D1	0.017	1.58

CBS	Cystathionine Beta-Synthase	0.029	1.58
C19orf84	Chromosome 19 Open Reading Frame 84	0.020	1.53
WNK3	WNK Lysine Deficient Protein Kinase 3	0.045	1.52
RGPD4	RANBP2 Like And GRIP Domain Containing 4	0.024	1.51
PLIN1	Perilipin 1	0.019	1.50
RNASE1	Ribonuclease A Family Member 1, Pancreatic	0.024	1.49
MEX3A	Mex-3 RNA Binding Family Member A	0.032	1.48
NFATC4	Nuclear Factor Of Activated T Cells 4	0.049	1.46
SUSD2	Sushi Domain Containing 2	0.005	1.46
OSCP1	Organic Solute Carrier Partner 1	0.023	1.41
IL9R	Interleukin 9 Receptor	0.028	1.38
TMEM262	Transmembrane Protein 262	0.035	1.38
FAM110C	Family With Sequence Similarity 110 Member C	0.017	1.36
CABYR	Calcium Binding Tyrosine Phosphorylation Regulated	0.041	1.36
CHDH	Choline Dehydrogenase	0.049	1.35
ZDHHC11B	Zinc Finger DHHC-Type Containing 11B	0.024	1.34
SGSM1	Small G Protein Signaling Modulator 1	< 0.001	1.34
SCN4B	Sodium Voltage-Gated Channel Beta Subunit 4	0.043	1.34
GRIN3B	Glutamate Ionotropic Receptor NMDA Type Subunit 3B	0.047	1.32
OR11G2	Olfactory Receptor Family 11 Subfamily G Member 2	0.027	1.31
GAS2	Growth Arrest Specific 2	0.022	1.30
CRHBP	Corticotropin Releasing Hormone Binding Protein	0.036	1.30
GLB1L2	Galactosidase Beta 1 Like 2	0.031	1.29
GALNT8	Polypeptide N-Acetylgalactosaminyltransferase 8	0.035	1.29
ZNF20	Zinc Finger Protein 20	0.017	1.28
ARHGEF25	Rho Guanine Nucleotide Exchange Factor 25	0.035	1.27
ZNF683	Zinc Finger Protein 683	0.017	1.27
RIBC1	RIB43A Domain With Coiled-Coils 1	0.022	1.27
ALPK3	Alpha Kinase 3	0.010	1.27
NTN5	Netrin 5	0.009	1.26

ZDHHC19	Zinc Finger DHHC-Type Palmitoyltransferase 19	0.039	1.26
RIPK4	Receptor Interacting Serine/Threonine Kinase 4	0.029	1.25
TGM1	Transglutaminase 1	0.044	1.24
NOXRED1	NADP Dependent Oxidoreductase Domain Containing 1	0.025	1.22
PRSS36	Serine Protease 36	0.022	1.21
SUV39H2	SUV39H2 Histone Lysine Methyltransferase	0.039	1.21
ZNF566	Zinc Finger Protein 566	0.009	1.20
ZNF135	Zinc Finger Protein 135	0.019	-1.21
H2BC11	H2B Clustered Histone 11	0.039	-1.21
POMC	Proopiomelanocortin	0.026	-1.21
ITGAV	Integrin Subunit Alpha V	0.044	-1.21
SLC22A23	Solute Carrier Family 22 Member 23	0.006	-1.22
NPRL3	NPR3 Like, GATOR1 Complex Subunit	0.030	-1.22
MRAS	Muscle RAS Oncogene Homolog	0.021	-1.23
FILIP1L	Filamin A Interacting Protein 1 Like	0.023	-1.23
PLEKHN1	Pleckstrin Homology Domain Containing N1	0.034	-1.23
NEURL1B	Neuralized E3 Ubiquitin Protein Ligase 1B	0.021	-1.23
SERPINE2	Serpin Family E Member 2	0.015	-1.24
SASH1	SAM And SH3 Domain Containing 1	0.022	-1.24
SLC6A8	Solute Carrier Family 6 Member 8	0.050	-1.24
TXNRD3	Thioredoxin Reductase 3	0.033	-1.24
PDGFC	Platelet Derived Growth Factor C	0.015	-1.25
UNC13B	Unc-13 Homolog B	0.021	-1.25
GUCY1A1	Guanylate Cyclase 1 Soluble Subunit Alpha 1	0.014	-1.25
NKD1	NKD Inhibitor Of WNT Signaling Pathway 1	0.036	-1.26
DIRAS1	DIRAS Family Gtpase 1	0.043	-1.26
ZRANB3	Zinc Finger RANBP2-Type Containing 3	0.043	-1.26
ACY3	Aminoacylase 3	0.021	-1.26
IQCK	IQ Motif Containing K	0.035	-1.26
CCDC62	Coiled-Coil Domain Containing 62	0.025	-1.26

SMARCA1	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, Subfamily A, Member 1	0.029	-1.26
EME1	Essential Meiotic Structure-Specific Endonuclease 1	0.027	-1.27
LRP5	LDL Receptor Related Protein 5	0.046	-1.27
CBX2	Chromobox 2	0.032	-1.27
FBXL16	F-Box And Leucine Rich Repeat Protein 16	0.021	-1.27
EBLN2	Endogenous Bornavirus Like Nucleoprotein 2	0.048	-1.28
SCNN1D	Sodium Channel Epithelial 1 Subunit Delta	0.004	-1.28
STXBP4	Syntaxin Binding Protein 4	0.027	-1.29
NPTX1	Neuronal Pentraxin 1	0.027	-1.29
BCAT1	Branched Chain Amino Acid Transaminase 1	0.036	-1.29
PCYT1B	Phosphate Cytidylyltransferase 1B, Choline	0.026	-1.30
TDRD9	Tudor Domain Containing 9	0.020	-1.30
RNFT2	Ring Finger Protein, Transmembrane 2	0.008	-1.31
DOCK1	Dedicator Of Cytokinesis 1	0.021	-1.31
LOXHD1	Lipoxygenase Homology PLAT Domains 1	0.035	-1.31
ANK2	Ankyrin 2	0.044	-1.32
SCN2A	Sodium Voltage-Gated Channel Alpha Subunit 2	0.037	-1.34
OR2W3	Olfactory Receptor Family 2 Subfamily W Member 3	0.023	-1.35
NRCAM	Neuronal Cell Adhesion Molecule	0.004	-1.35
SOX7	SRY-Box Transcription Factor 7	0.030	-1.36
FGFR4	Fibroblast Growth Factor Receptor 4	0.012	-1.36
LRRC36	Leucine Rich Repeat Containing 36	0.021	-1.37
APBA1	Amyloid Beta Precursor Protein Binding Family A Member 1	0.009	-1.37
SLC7A9	Solute Carrier Family 7 Member 9	0.019	-1.38
ELAVL4	ELAV Like RNA Binding Protein 4	0.019	-1.38
CELF3	CUGBP Elav-Like Family Member 3	0.038	-1.38
MYLPF	Myosin Light Chain, Phosphorylatable, Fast Skeletal Muscle	0.022	-1.38
CAV1	Caveolin 1	0.031	-1.38
ERG	ETS Transcription Factor ERG	0.044	-1.38

CC2D2A	Coiled-Coil And C2 Domain Containing 2A	0.015	-1.39
PRKG2	Protein Kinase Cgmp-Dependent 2	0.045	-1.39
FGF9	Fibroblast Growth Factor 9	0.039	-1.39
ARHGAP20	Rho Gtpase Activating Protein 20	0.039	-1.39
BNC2	Basonuclin 2	0.023	-1.39
MCF2L2	MCF.2 Cell Line Derived Transforming Sequence-Like 2	0.008	-1.39
CD1B	CD1b Molecule	0.017	-1.39
GRB14	Growth Factor Receptor Bound Protein 14	0.024	-1.40
LHFPL6	LHFPL Tetraspan Subfamily Member 6	0.020	-1.41
SLC8A2	Solute Carrier Family 8 Member A2	0.039	-1.41
TTC28	Tetratricopeptide Repeat Domain 28	0.005	-1.42
GRM5	Glutamate Metabotropic Receptor 5	0.049	-1.43
SEC14L4	SEC14 Like Lipid Binding 4	0.022	-1.43
NOVA1	NOVA Alternative Splicing Regulator 1	0.044	-1.44
CHRFAM7A	CHRNA7 (Exons 5-10) And FAM7A (Exons A-E) Fusion	0.020	-1.44
ITLN1	Intelectin 1	0.011	-1.45
ACTG2	Actin Gamma 2, Smooth Muscle	0.018	-1.45
TTLL10	Tubulin Tyrosine Ligase Like 10	0.038	-1.46
CADPS2	Calcium Dependent Secretion Activator 2	0.041	-1.47
STARD13	Star Related Lipid Transfer Domain Containing 13	0.009	-1.49
PDE1A	Phosphodiesterase 1A	0.038	-1.49
TLN2	Talin 2	0.017	-1.49
RBPMS2	RNA Binding Protein, Mrna Processing Factor 2	0.034	-1.51
FJX1	Four-Jointed Box Kinase 1	0.033	-1.52
CTTNBP2	Cortactin Binding Protein 2	0.011	-1.52
MMP21	Matrix Metallopeptidase 21	0.037	-1.53
LY6G6C	Lymphocyte Antigen 6 Family Member G6C	0.041	-1.53
RAI14	Retinoic Acid Induced 14	0.030	-1.54
VSTM2B	V-Set And Transmembrane Domain Containing 2B	0.044	-1.54

TUBB3	Tubulin Beta 3 Class III	0.005	-1.55
AREG	Amphiregulin	0.021	-1.56
PRL	Prolactin	0.045	-1.57
NTRK2	Neurotrophic Receptor Tyrosine Kinase 2	0.001	-1.58
KRT2	Keratin 2	0.003	-1.58
CHSY3	Chondroitin Sulfate Synthase 3	0.038	-1.59
C4orf36	Chromosome 4 Open Reading Frame 36	0.042	-1.61
DYNLT5	Dynein Light Chain Tctex-Type Family Member 5	0.027	-1.63
DPYSL4	Dihydropyrimidinase Like 4	0.010	-1.63
HOXA5	Homeobox A5	0.020	-1.66
AK7	Adenylate Kinase 7	0.034	-1.66
SCHIP1	Schwannomin Interacting Protein 1	0.020	-1.66
RALYL	RALY RNA Binding Protein Like	0.019	-1.67
AFAP1L1	Actin Filament Associated Protein 1 Like 1	0.002	-1.69
KCNQ2	Potassium Voltage-Gated Channel Subfamily Q Member 2	0.037	-1.70
SKIDA1	SKI/DACH Domain Containing 1	0.023	-1.70
GLI3	GLI Family Zinc Finger 3	0.020	-1.77
H3C13	H3 Clustered Histone 13	0.042	-1.77
KCNJ10	Potassium Inwardly Rectifying Channel Subfamily J Member 10	0.011	-1.81
MEDAG	Mesenteric Estrogen Dependent Adipogenesis	0.037	-1.81
LURAP1L	Leucine Rich Adaptor Protein 1 Like	0.009	-1.83
CCL25	C-C Motif Chemokine Ligand 25	0.034	-1.83
ATRNL1	Attractin Like 1	0.003	-1.86
ENTPD3	Ectonucleoside Triphosphate Diphosphohydrolase 3	0.041	-1.87
ZBED9	Zinc Finger BED-Type Containing 9	0.023	-1.88
SOX11	SRY-Box Transcription Factor 11	0.030	-1.90
MRGPRE	MAS Related GPR Family Member E	0.048	-1.91
AIF1L	Allograft Inflammatory Factor 1 Like	0.011	-1.91
HECW1	HECT, C2 And WW Domain Containing E3 Ubiquitin Protein Ligase 1	0.032	-1.91

DNAH14	Dynein Axonemal Heavy Chain 14	0.041	-1.94
MYO18B	Myosin XVIIIB	0.032	-1.95
DOK6	Docking Protein 6	0.017	-1.96
CLVS1	Clavesin 1	0.008	-2.02
MUC16	Mucin 16, Cell Surface Associated	0.016	-2.04
DGKI	Diacylglycerol Kinase Iota	0.012	-2.09
GLP1R	Glucagon Like Peptide 1 Receptor	0.005	-2.14
C8orf34	Chromosome 8 Open Reading Frame 34	0.008	-2.15
CCDC144A	Coiled-Coil Domain Containing 144A	0.008	-2.15
SPHKAP	SPHK1 Interactor, AKAP Domain Containing	0.026	-2.19
CHRD	Chordin	0.019	-2.33
ARID3C	AT-Rich Interaction Domain 3C	0.015	-2.36
TFAP2A	Transcription Factor AP-2 Alpha	0.050	-2.44
PTH1R	Parathyroid Hormone 1 Receptor	0.022	-2.45
PLEKHS1	Pleckstrin Homology Domain Containing S1	0.002	-2.52
MMP1	Matrix Metallopeptidase 1	0.004	-3.13
TBC1D3F	TBC1 Domain Family Member 3F	0.012	-5.03
TBC1D3H	TBC1 Domain Family Member 3H	0.002	-80.56
TBC1D3G	TBC1 Domain Family Member 3G	0.002	-355.26

Table 4E. List of 310 genes differently modulated in males MDD vs males HR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3G	TBC1 Domain Family Member 3G	< 0.001	4963.1
TBC1D3	TBC1 Domain Family Member 3	0.023	10.86
ADARB2	Adenosine Deaminase RNA Specific B2 (Inactive)	0.003	9.15
PTP4A1	Protein Tyrosine Phosphatase 4A1	0.033	7.24
ITGA8	Integrin Subunit Alpha 8	0.008	3.72
TGIF2-RAB5IF	TGIF2-RAB5IF Readthrough	0.025	3.68
CHURC1-FNTB	CHURC1-FNTB Readthrough	0.043	3.64
POC1B-GALNT4	POC1B-GALNT4 Readthrough	0.033	3.53
KLHL41	Kelch Like Family Member 41	0.010	3.44
ADAMTS2	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 2	0.002	3.08
POU5F2	POU Domain Class 5, Transcription Factor 2	0.002	2.84
DCN	Decorin	0.009	2.78
C16orf46	Chromosome 16 Open Reading Frame 46	0.038	2.76
DGKI	Diacylglycerol Kinase Iota	0.008	2.72
MEIKIN	Meiotic Kinetochore Factor	0.029	2.67
PRR15	Proline Rich 15	0.027	2.59
SLC13A4	Solute Carrier Family 13 Member 4	0.022	2.51
SLC5A11	Solute Carrier Family 5 Member 11	0.016	2.49
PSMB11	Proteasome Subunit Beta 11	0.023	2.43
GLI3	GLI Family Zinc Finger 3	0.023	2.40
VWDE	Von Willebrand Factor D And EGF Domains	0.029	2.31
GPRC5D	G Protein-Coupled Receptor Class C Group 5 Member D	0.006	2.26
PKP3	Plakophilin 3	0.018	2.26
LHFPL5	LHFPL Tetraspan Subfamily Member 5	0.030	2.22

COL1A2	Collagen Type I Alpha 2 Chain	0.036	2.20
LRRTM3	Leucine Rich Repeat Transmembrane Neuronal 3	0.006	2.16
GLDC	Glycine Decarboxylase	0.002	2.11
SDC1	Syndecan 1	0.028	2.05
CD177	CD177 Molecule	0.029	1.99
TERT	Telomerase Reverse Transcriptase	0.026	1.99
ATP2C2	Atpase Secretory Pathway Ca2+ Transporting 2	0.049	1.98
MIXL1	Mix Paired-Like Homeobox	0.007	1.96
NACAD	NAC Alpha Domain Containing	0.024	1.95
PADI6	Peptidyl Arginine Deiminase 6	0.006	1.95
SLITRK3	SLIT And NTRK Like Family Member 3	0.012	1.91
C1QC	Complement C1q C Chain	0.018	1.88
A3GALT2	Alpha 1,3-Galactosyltransferase 2	0.041	1.86
NLGN4Y	Neuroligin 4 Y-Linked	0.038	1.84
DNAH17	Dynein Axonemal Heavy Chain 17	0.044	1.83
SCHIP1	Schwannomin Interacting Protein 1	0.042	1.82
DRC1	Dynein Regulatory Complex Subunit 1	0.044	1.79
CDC25A	Cell Division Cycle 25A	0.007	1.79
HEY1	Hes Related Family Bhlh Transcription Factor With YRPW Motif 1	0.019	1.79
SKA3	Spindle And Kinetochore Associated Complex Subunit 3	0.004	1.78
KCNMA1	Potassium Calcium-Activated Channel Subfamily M Alpha 1	0.020	1.77
POLQ	DNA Polymerase Theta	0.004	1.76
TBC1D8B	TBC1 Domain Family Member 8B	0.008	1.76
CHRFAM7A	CHRNA7 (Exons 5-10) And FAM7A (Exons A-E) Fusion	0.003	1.75
KNL1	Kinetochore Scaffold 1	0.011	1.73
C3orf52	Chromosome 3 Open Reading Frame 52	0.018	1.73
CDC20	Cell Division Cycle 20	0.019	1.72
BHLHA15	Basic Helix-Loop-Helix Family Member A15	0.028	1.71

ARNTL2	Aryl Hydrocarbon Receptor Nuclear Translocator Like 2	0.005	1.71
LSAMP	Limbic System Associated Membrane Protein	0.039	1.70
MZB1	Marginal Zone B And B1 Cell Specific Protein	0.011	1.69
BMP8A	Bone Morphogenetic Protein 8a	0.042	1.68
PRG4	Proteoglycan 4	0.011	1.66
TMEM255A	Transmembrane Protein 255A	0.035	1.66
HTR3B	5-Hydroxytryptamine Receptor 3B	0.041	1.64
ERCC6L	ERCC Excision Repair 6 Like, Spindle Assembly Checkpoint Helicase	0.032	1.64
COL5A3	Collagen Type V Alpha 3 Chain	0.043	1.63
PHYHD1	Phytanoyl-Coa Dioxygenase Domain Containing 1	0.006	1.63
FAM187A	Family With Sequence Similarity 187 Member A	0.022	1.63
RAP1GAP	RAP1 Gtpase Activating Protein	0.028	1.62
ASPM	Assembly Factor For Spindle Microtubules	0.017	1.62
KIFC1	Kinesin Family Member C1	0.003	1.62
TTBK1	Tau Tubulin Kinase 1	0.034	1.61
FFAR3	Free Fatty Acid Receptor 3	0.001	1.61
CENPF	Centromere Protein F	0.042	1.60
TXNDC5	Thioredoxin Domain Containing 5	0.014	1.60
BAMBI	BMP And Activin Membrane Bound Inhibitor	0.015	1.60
POLN	DNA Polymerase Nu	0.013	1.60
ESPL1	Extra Spindle Pole Bodies Like 1, Separase	0.021	1.59
SH3PXD2B	SH3 And PX Domains 2B	0.008	1.58
RRM2	Ribonucleotide Reductase Regulatory Subunit M2	0.050	1.58
CLSPN	Claspin	0.008	1.57
FRMD3	FERM Domain Containing 3	0.001	1.56
ARHGAP23	Rho Gtpase Activating Protein 23	0.031	1.56
KIF4A	Kinesin Family Member 4A	0.030	1.55
CDCP1	CUB Domain Containing Protein 1	0.031	1.53

TK1	Thymidine Kinase 1	0.007	1.52
KIF18B	Kinesin Family Member 18B	0.047	1.51
EME1	Essential Meiotic Structure-Specific Endonuclease 1	0.025	1.51
BUB1	BUB1 Mitotic Checkpoint Serine/Threonine Kinase	0.017	1.50
LOXHD1	Lipoxygenase Homology PLAT Domains 1	0.011	1.50
RSPH9	Radial Spoke Head Component 9	0.039	1.49
COCH	Cochlin	0.009	1.47
PKMYT1	Protein Kinase, Membrane Associated Tyrosine/Threonine 1	0.008	1.47
SEMA3G	Semaphorin 3G	0.021	1.47
UNC13B	Unc-13 Homolog B	0.004	1.45
GTSE1	G2 And S-Phase Expressed 1	0.009	1.45
DERL3	Derlin 3	0.036	1.45
RIPOR3	RIPOR Family Member 3	0.023	1.43
COL4A4	Collagen Type IV Alpha 4 Chain	0.046	1.42
IQCK	IQ Motif Containing K	0.036	1.41
ARL6	ADP Ribosylation Factor Like Gtpase 6	0.002	1.41
TPX2	TPX2 Microtubule Nucleation Factor	0.030	1.41
RGS16	Regulator Of G Protein Signaling 16	0.009	1.40
KLK1	Kallikrein 1	0.043	1.39
RECQL4	Recq Like Helicase 4	0.015	1.39
AURKB	Aurora Kinase B	0.004	1.39
B9D1	B9 Domain Containing 1	0.032	1.39
CD38	CD38 Molecule	0.033	1.37
ANGPTL6	Angiopoietin Like 6	0.015	1.37
DAAM1	Dishevelled Associated Activator Of Morphogenesis 1	0.001	1.37
KIF24	Kinesin Family Member 24	0.018	1.36
BMP6	Bone Morphogenetic Protein 6	0.001	1.36
SERPINE1	Serpin Family E Member 1	0.044	1.36
H4C15	H4 Clustered Histone 15	0.040	1.35
VWA7	Von Willebrand Factor A Domain Containing 7	0.015	1.35

GPT2	Glutamic--Pyruvic Transaminase 2	0.006	1.34
NUGGC	Nuclear Gtpase, Germinal Center Associated	0.007	1.34
BCAR3	BCAR3 Adaptor Protein, NSP Family Member	0.018	1.33
PLK1	Polo Like Kinase 1	0.011	1.33
ELL2	Elongation Factor For RNA Polymerase II 2	0.007	1.33
ORC1	Origin Recognition Complex Subunit 1	0.047	1.32
ALG10	ALG10 Alpha-1,2-Glucosyltransferase	0.005	1.32
ZNF107	Zinc Finger Protein 107	0.029	1.31
ARRDC4	Arrestin Domain Containing 4	0.013	1.31
QRICH2	Glutamine Rich 2	0.011	1.30
ADM	Adrenomedullin	0.049	1.30
ZDHHC21	Zinc Finger DHHC-Type Palmitoyltransferase 21	0.016	1.30
KIF11	Kinesin Family Member 11	0.038	1.29
PDGFB	Platelet Derived Growth Factor Subunit B	0.008	1.29
MYO1D	Myosin ID	0.024	1.29
MFSD14B	Major Facilitator Superfamily Domain Containing 14B	0.002	1.29
CHPF	Chondroitin Polymerizing Factor	0.019	1.28
UHRF1	Ubiquitin Like With PHD And Ring Finger Domains 1	0.048	1.28
ARL13B	ADP Ribosylation Factor Like Gtpase 13B	0.011	1.26
SLC44A1	Solute Carrier Family 44 Member 1	0.002	1.26
EHD3	EH Domain Containing 3	0.001	1.26
EPHB1	EPH Receptor B1	0.039	1.26
TENT5C	Terminal Nucleotidyltransferase 5C	0.047	1.25
KRTCAP3	Keratinocyte Associated Protein 3	0.050	1.25
MAP3K7CL	MAP3K7 C-Terminal Like	0.028	1.24
MISP3	MISP Family Member 3	0.011	1.24
SLC1A4	Solute Carrier Family 1 Member 4	0.043	1.24
P2RX1	Purinergic Receptor P2X 1	0.040	1.23
UAP1	UDP-N-Acetylglucosamine Pyrophosphorylase 1	0.028	1.23
FBXO22	F-Box Protein 22	0.016	1.22

FKBP11	FKBP Prolyl Isomerase 11	0.022	1.22
LRFN3	Leucine Rich Repeat And Fibronectin Type III Domain Containing 3	0.045	1.22
ASNS	Asparagine Synthetase (Glutamine-Hydrolyzing)	0.035	1.21
SMIM13	Small Integral Membrane Protein 13	0.015	1.21
MMD	Monocyte To Macrophage Differentiation Associated	0.024	1.21
FHIP2A	FHF Complex Subunit HOOK Interacting Protein 2A	0.014	1.21
NCOA7	Nuclear Receptor Coactivator 7	0.044	1.21
NOTCH2NLA	Notch 2 N-Terminal Like A	0.030	1.21
ABCB6	ATP Binding Cassette Subfamily B Member 6 (Langereis Blood Group)	0.049	1.20
SLC33A1	Solute Carrier Family 33 Member 1	0.001	1.20
PI4K2B	Phosphatidylinositol 4-Kinase Type 2 Beta	0.027	1.20
SH3PXD2A	SH3 And PX Domains 2A	0.036	-1.20
NAAA	N-Acylethanolamine Acid Amidase	0.038	-1.20
EPHX2	Epoxide Hydrolase 2	0.037	-1.21
HEATR5A	HEAT Repeat Containing 5A	0.038	-1.21
CDC42BPB	CDC42 Binding Protein Kinase Beta	0.044	-1.21
WDR27	WD Repeat Domain 27	0.032	-1.21
TSSK6	Testis Specific Serine Kinase 6	0.024	-1.21
TRIP10	Thyroid Hormone Receptor Interactor 10	0.045	-1.21
ATPSCKMT	ATP Synthase C Subunit Lysine N-Methyltransferase	0.025	-1.21
POPDC2	Popeye Domain Containing 2	0.029	-1.22
ICA1	Islet Cell Autoantigen 1	0.030	-1.22
AOPEP	Aminopeptidase O (Putative)	0.009	-1.23
TOGARAM1	TOG Array Regulator Of Axonemal Microtubules 1	0.037	-1.23
DNAJC16	Dnaj Heat Shock Protein Family (Hsp40) Member C16	0.013	-1.23
LILRB1	Leukocyte Immunoglobulin Like Receptor B1	0.017	-1.23
CRTAM	Cytotoxic And Regulatory T Cell Molecule	0.021	-1.24

CSPP1	Centrosome And Spindle Pole Associated Protein 1	< 0.001	-1.24
GEMIN2	Gem Nuclear Organelle Associated Protein 2	0.049	-1.24
TMEM256	Transmembrane Protein 256	0.035	-1.24
RPS28	Ribosomal Protein S28	0.031	-1.25
ITM2A	Integral Membrane Protein 2A	0.035	-1.25
LIN7B	Lin-7 Homolog B, Crumbs Cell Polarity Complex Component	0.021	-1.26
ZNF528	Zinc Finger Protein 528	0.004	-1.26
FER	FER Tyrosine Kinase	0.023	-1.26
PASK	PAS Domain Containing Serine/Threonine Kinase	0.018	-1.27
HDGFL3	HDGF Like 3	0.042	-1.27
TPPP3	Tubulin Polymerization Promoting Protein Family Member 3	0.026	-1.27
PLXDC1	Plexin Domain Containing 1	0.041	-1.27
ADAM22	ADAM Metallopeptidase Domain 22	0.023	-1.28
PPP1R13L	Protein Phosphatase 1 Regulatory Subunit 13 Like	0.019	-1.28
IGSF9B	Immunoglobulin Superfamily Member 9B	0.036	-1.28
GTPBP10	GTP Binding Protein 10	0.027	-1.28
ATG9B	Autophagy Related 9B	0.010	-1.28
TRIM73	Tripartite Motif Containing 73	0.029	-1.29
UST	Uronyl 2-Sulfotransferase	0.007	-1.29
MICALL2	MICAL Like 2	0.011	-1.30
RPS15A	Ribosomal Protein S15a	0.034	-1.30
ADGRA3	Adhesion G Protein-Coupled Receptor A3	0.025	-1.30
CFAP44	Cilia And Flagella Associated Protein 44	0.019	-1.31
PSPH	Phosphoserine Phosphatase	0.017	-1.31
AMOTL1	Angiomotin Like 1	0.016	-1.32
SEZ6L	Seizure Related 6 Homolog Like	0.027	-1.32
FAM20C	FAM20C Golgi Associated Secretory Pathway Kinase	0.026	-1.32
NAP1L3	Nucleosome Assembly Protein 1 Like 3	0.007	-1.33
RAB3IP	RAB3A Interacting Protein	0.003	-1.33
ZC3H12B	Zinc Finger CCCH-Type Containing 12B	0.027	-1.33

NT5C3B	5'-Nucleotidase, Cytosolic IIIB	0.004	-1.34
BTBD19	BTB Domain Containing 19	0.026	-1.34
ACOT13	Acyl-Coa Thioesterase 13	0.003	-1.35
ADHFE1	Alcohol Dehydrogenase Iron Containing 1	0.002	-1.35
NIPSNAP3B	Nipsnap Homolog 3B	0.020	-1.36
PPM1J	Protein Phosphatase, Mg2+/Mn2+ Dependent 1J	0.022	-1.36
CUX2	Cut Like Homeobox 2	0.010	-1.37
ATP1A3	Atpase Na+/K+ Transporting Subunit Alpha 3	0.025	-1.37
NPIPB2	Nuclear Pore Complex Interacting Protein Family Member B2	0.021	-1.37
PTPRN	Protein Tyrosine Phosphatase Receptor Type N	0.044	-1.38
ARHGEF25	Rho Guanine Nucleotide Exchange Factor 25	0.030	-1.39
SUSD4	Sushi Domain Containing 4	0.012	-1.39
ADGRD1	Adhesion G Protein-Coupled Receptor D1	0.031	-1.39
NPIPB13	Nuclear Pore Complex Interacting Protein Family, Member B13	0.029	-1.39
ZNF177	Zinc Finger Protein 177	0.046	-1.39
SERF1A	Small EDRK-Rich Factor 1A	0.014	-1.39
KANK2	KN Motif And Ankyrin Repeat Domains 2	0.043	-1.39
C16orf87	Chromosome 16 Open Reading Frame 87	0.014	-1.40
EPB41L1	Erythrocyte Membrane Protein Band 4.1 Like 1	0.047	-1.40
CERCAM	Cerebral Endothelial Cell Adhesion Molecule	0.016	-1.41
KCNE5	Potassium Voltage-Gated Channel Subfamily E Regulatory Subunit 5	0.021	-1.41
GAS1	Growth Arrest Specific 1	0.045	-1.42
PLLP	Plasmolipin	0.015	-1.43
ARL17A	ADP Ribosylation Factor Like Gtpase 17A	0.014	-1.43
ULK4	Unc-51 Like Kinase 4	0.016	-1.43
NRIP3	Nuclear Receptor Interacting Protein 3	0.009	-1.43
ANKUB1	Ankyrin Repeat And Ubiquitin Domain Containing 1	0.047	-1.43
LZTS1	Leucine Zipper Tumor Suppressor 1	0.038	-1.44

NTN5	Netrin 5	0.026	-1.44
UPK3B	Uroplakin 3B	0.045	-1.44
DCST2	DC-STAMP Domain Containing 2	0.035	-1.46
TMEM182	Transmembrane Protein 182	0.012	-1.46
ZP3	Zona Pellucida Glycoprotein 3	0.012	-1.47
PLA2G2D	Phospholipase A2 Group IID	0.037	-1.48
RYR1	Ryanodine Receptor 1	0.019	-1.48
PCDHGA6	Protocadherin Gamma Subfamily A, 6	0.030	-1.49
PGLYRP2	Peptidoglycan Recognition Protein 2	0.038	-1.51
CD34	CD34 Molecule	0.017	-1.52
SFTPD	Surfactant Protein D	0.009	-1.56
ZNF391	Zinc Finger Protein 391	0.003	-1.56
SHD	Src Homology 2 Domain Containing Transforming Protein D	0.032	-1.57
RHCE	Rh Blood Group Ccee Antigens	0.029	-1.59
PDZD2	PDZ Domain Containing 2	0.043	-1.59
SPTSSB	Serine Palmitoyltransferase Small Subunit B	0.020	-1.60
GARNL3	Gtpase Activating Rap/Rangap Domain Like 3	0.013	-1.60
ZDHHC11B	Zinc Finger DHHC-Type Containing 11B	0.009	-1.61
PRR36	Proline Rich 36	0.037	-1.61
TRNP1	TMF1 Regulated Nuclear Protein 1	0.004	-1.62
MYO1B	Myosin IB	0.009	-1.63
CEMP1	Cementum Protein 1	0.028	-1.63
MXRA8	Matrix Remodeling Associated 8	0.009	-1.64
HACD1	3-Hydroxyacyl-Coa Dehydratase 1	0.008	-1.66
PFDN4	Prefoldin Subunit 4	0.009	-1.67
ALDH7A1	Aldehyde Dehydrogenase 7 Family Member A1	0.021	-1.68
CCDC171	Coiled-Coil Domain Containing 171	0.043	-1.69
KCNK1	Potassium Two Pore Domain Channel Subfamily K Member 1	0.049	-1.70
PLS3	Plastin 3	0.010	-1.71
TLCD1	TLC Domain Containing 1	0.015	-1.72

ASIC2	Acid Sensing Ion Channel Subunit 2	0.042	-1.72
COLEC12	Collectin Subfamily Member 12	0.009	-1.72
ABCA6	ATP Binding Cassette Subfamily A Member 6	0.018	-1.73
CDHR5	Cadherin Related Family Member 5	0.025	-1.76
GALNT9	Polypeptide N-Acetylgalactosaminyltransferase 9	0.027	-1.77
TNS2	Tensin 2	0.009	-1.77
PLIN1	Perilipin 1	0.025	-1.78
MAGI2	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2	0.024	-1.79
KIAA1549	Kiaa1549	0.029	-1.80
MATN1	Matrilin 1	0.036	-1.83
ROBO1	Roundabout Guidance Receptor 1	0.001	-1.85
LRP6	LDL Receptor Related Protein 6	< 0.001	-1.86
ANKRD18A	Ankyrin Repeat Domain 18A	0.043	-1.87
FOLR3	Folate Receptor Gamma	0.013	-1.87
NFILZ	NFIL3 Like Basic Leucine Zipper	0.009	-1.87
SFRP5	Secreted Frizzled Related Protein 5	0.009	-1.90
STXBP6	Syntaxin Binding Protein 6	0.044	-1.92
NKAIN2	Sodium/Potassium Transporting Atpase Interacting 2	0.023	-1.96
NPC1L1	NPC1 Like Intracellular Cholesterol Transporter 1	0.045	-1.96
PLGLB2	Plasminogen Like B2	0.001	-1.97
BCL2L14	BCL2 Like 14	0.021	-1.99
SHC3	SHC Adaptor Protein 3	0.044	-1.99
SLC7A3	Solute Carrier Family 7 Member 3	0.027	-1.99
NECAB1	N-Terminal EF-Hand Calcium Binding Protein 1	0.030	-1.99
ZNF704	Zinc Finger Protein 704	0.042	-2.01
DRC3	Dynein Regulatory Complex Subunit 3	0.013	-2.05
SIGLEC11	Sialic Acid Binding Ig Like Lectin 11	0.005	-2.05
SLC6A20	Solute Carrier Family 6 Member 20	0.035	-2.07
TAC3	Tachykinin Precursor 3	0.035	-2.12

HOXA7	Homeobox A7	0.019	-2.19
PLG	Plasminogen	0.019	-2.19
CNNM1	Cyclin And CBS Domain Divalent Metal Cation Transport Mediator 1	0.003	-2.23
BICC1	Bicc Family RNA Binding Protein 1	0.008	-2.25
LPL	Lipoprotein Lipase	< 0.001	-2.39
DNAH2	Dynein Axonemal Heavy Chain 2	0.035	-2.41
SUMO4	Small Ubiquitin Like Modifier 4	0.010	-2.43
CEMIP	Cell Migration Inducing Hyaluronidase 1	0.032	-2.44
VANGL2	VANGL Planar Cell Polarity Protein 2	0.007	-2.47
IGSF11	Immunoglobulin Superfamily Member 11	0.001	-2.58
GSTM5	Glutathione S-Transferase Mu 5	0.002	-2.60
GSTT2B	Glutathione S-Transferase Theta 2B	0.048	-2.66
LEKR1	Leucine, Glutamate And Lysine Rich 1	0.010	-2.66
CBLN2	Cerebellin 2 Precursor	0.008	-2.76
DNAH11	Dynein Axonemal Heavy Chain 11	0.021	-2.89
HBZ	Hemoglobin Subunit Zeta	0.002	-3.20
CHST6	Carbohydrate Sulfotransferase 6	0.009	-3.22
C4BPA	Complement Component 4 Binding Protein Alpha	0.001	-3.44
ZSCAN23	Zinc Finger And SCAN Domain Containing 23	0.003	-3.83
SYPL2	Synaptophysin Like 2	0.002	-3.90
PNMA8B	PNMA Family Member 8B	< 0.001	-3.94
POU2F3	POU Class 2 Homeobox 3	0.004	-4.04
OR7D2	Olfactory Receptor Family 7 Subfamily D Member 2	0.020	-4.11
PNMA8A	PNMA Family Member 8A	0.004	-11.80
CYP26B1	Cytochrome P450 Family 26 Subfamily B Member 1	0.001	-16.46
TBC1D3H	TBC1 Domain Family Member 3H	< 0.001	-2507.3

Table 5E. List of 377 genes differently modulated in males MDD vs males LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05).

Gene Symbol	Gene Assignment	p-value	Fold-Change
DEFA1	Defensin Alpha 1	< 0.001	15.06
ADARB2	Adenosine Deaminase RNA Specific B2 (Inactive)	0.001	11.41
PRR15	Proline Rich 15	< 0.001	5.92
AMPD1	Adenosine Monophosphate Deaminase 1	0.014	3.76
SRGAP1	SLIT-ROBO Rho Gtpase Activating Protein 1	< 0.001	3.69
TTC23L	Tetratricopeptide Repeat Domain 23 Like	0.037	3.66
ITGA8	Integrin Subunit Alpha 8	0.011	3.65
DCN	Decorin	0.007	3.44
CCDC68	Coiled-Coil Domain Containing 68	0.021	3.31
SHISA3	Shisa Family Member 3	0.019	3.21
IGF1	Insulin Like Growth Factor 1	0.013	3.14
ESRRB	Estrogen Related Receptor Beta	0.018	3.10
ZYG11A	Zyg-11 Family Member A, Cell Cycle Regulator	0.016	3.06
OLFM4	Olfactomedin 4	< 0.001	3.03
RGS13	Regulator Of G Protein Signaling 13	0.014	2.98
FZD7	Frizzled Class Receptor 7	< 0.001	2.86
EYA2	EYA Transcriptional Coactivator And Phosphatase 2	0.003	2.77
SDC1	Syndecan 1	0.003	2.77
SLC44A5	Solute Carrier Family 44 Member 5	0.037	2.72
NLGN4Y	Neuroligin 4 Y-Linked	0.002	2.72
PLAAT2	Phospholipase A And Acyltransferase 2	0.005	2.70
C9orf152	Chromosome 9 Open Reading Frame 152	0.047	2.67
CPS1	Carbamoyl-Phosphate Synthase 1	0.003	2.66
SCUBE2	Signal Peptide, CUB Domain And EGF Like Domain Containing 2	0.004	2.66
ZNF474	Zinc Finger Protein 474	0.041	2.58
SH2D5	SH2 Domain Containing 5	0.038	2.56

MEIS3	Meis Homeobox 3	0.032	2.53
TP63	Tumor Protein P63	< 0.001	2.51
RHOD	Ras Homolog Family Member D	0.012	2.47
GPRC5D	G Protein-Coupled Receptor Class C Group 5 Member D	0.007	2.45
MET	MET Proto-Oncogene, Receptor Tyrosine Kinase	0.047	2.41
BMP8A	Bone Morphogenetic Protein 8a	0.002	2.38
PRSS8	Serine Protease 8	0.021	2.28
FAM149A	Family With Sequence Similarity 149 Member A	0.028	2.26
PPARG	Peroxisome Proliferator Activated Receptor Gamma	0.009	2.19
BHLHA15	Basic Helix-Loop-Helix Family Member A15	0.004	2.19
GLDC	Glycine Decarboxylase	0.004	2.19
VEGFC	Vascular Endothelial Growth Factor C	0.005	2.15
MIXL1	Mix Paired-Like Homeobox	0.009	2.13
HTRA3	Htra Serine Peptidase 3	0.011	2.13
LTF	Lactotransferrin	0.005	2.09
DEPDC1	DEP Domain Containing 1	0.014	2.07
DNAH17	Dynein Axonemal Heavy Chain 17	0.013	2.07
TSPO2	Translocator Protein 2	0.009	2.05
MTUS2	Microtubule Associated Scaffold Protein 2	0.042	2.04
SPATA1	Spermatogenesis Associated 1	0.005	2.01
JCHAIN	Joining Chain Of Multimeric Iga And Igm	0.010	2.00
TERT	Telomerase Reverse Transcriptase	0.018	1.99
TROAP	Trophinin Associated Protein	< 0.001	1.99
COCH	Cochlin	< 0.001	1.98
GAREM1	GRB2 Associated Regulator Of MAPK1 Subtype 1	0.033	1.98
SDS	Serine Dehydratase	0.003	1.95
APOBEC3B	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3B	0.019	1.94
DEFA4	Defensin Alpha 4	0.044	1.94
ABCG2	ATP Binding Cassette Subfamily G Member 2 (Junior Blood Group)	0.003	1.92

MZB1	Marginal Zone B And B1 Cell Specific Protein	0.004	1.91
NECTIN2	Nectin Cell Adhesion Molecule 2	0.016	1.90
PAQR5	Progestin And Adipoq Receptor Family Member 5	0.017	1.90
VAT1L	Vesicle Amine Transport 1 Like	0.046	1.89
CEACAM6	CEA Cell Adhesion Molecule 6	0.018	1.89
TXNDC5	Thioredoxin Domain Containing 5	0.003	1.89
AP3B2	Adaptor Related Protein Complex 3 Subunit Beta 2	0.033	1.89
ZBED6	Zinc Finger BED-Type Containing 6	0.019	1.88
CRISP3	Cysteine Rich Secretory Protein 3	0.001	1.88
KCNJ3	Potassium Inwardly Rectifying Channel Subfamily J Member 3	0.037	1.88
MMP8	Matrix Metallopeptidase 8	0.028	1.87
KCNN3	Potassium Calcium-Activated Channel Subfamily N Member 3	0.034	1.86
LOC112267968	Uncharacterized LOC112267968	0.037	1.86
TNFRSF17	TNF Receptor Superfamily Member 17	0.024	1.85
ILDR2	Immunoglobulin Like Domain Containing Receptor 2	0.038	1.84
E2F8	E2F Transcription Factor 8	0.017	1.83
PPARGC1A	PPARG Coactivator 1 Alpha	0.006	1.82
LIPH	Lipase H	0.026	1.81
CHAD	Chondroadherin	0.001	1.80
CDC25A	Cell Division Cycle 25A	0.017	1.80
ALG14	ALG14 UDP-N-Acetylglucosaminyltransferase Subunit	0.015	1.79
TP73	Tumor Protein P73	0.013	1.79
E2F7	E2F Transcription Factor 7	0.019	1.79
IGLL5	Immunoglobulin Lambda Like Polypeptide 5	0.020	1.78
ZNF521	Zinc Finger Protein 521	0.039	1.77
CEACAM8	CEA Cell Adhesion Molecule 8	0.045	1.76
CRHBP	Corticotropin Releasing Hormone Binding Protein	0.003	1.76
SLC28A3	Solute Carrier Family 28 Member 3	0.027	1.75
ELANE	Elastase, Neutrophil Expressed	0.022	1.75

RASSF6	Ras Association Domain Family Member 6	0.008	1.74
ADGRG6	Adhesion G Protein-Coupled Receptor G6	0.039	1.74
HPDL	4-Hydroxyphenylpyruvate Dioxygenase Like	0.006	1.72
DTL	Denticleless E3 Ubiquitin Protein Ligase Homolog	0.013	1.71
KIF4A	Kinesin Family Member 4A	0.020	1.71
POLN	DNA Polymerase Nu	0.006	1.70
DZIP1L	DAZ Interacting Zinc Finger Protein 1 Like	0.032	1.69
RNASE2	Ribonuclease A Family Member 2	0.027	1.69
CDC20	Cell Division Cycle 20	0.019	1.69
PRUNE2	Prune Homolog 2 With BCH Domain	0.045	1.68
PLIN1	Perilipin 1	0.023	1.68
ESPL1	Extra Spindle Pole Bodies Like 1, Separase	0.009	1.67
BUB1B	BUB1 Mitotic Checkpoint Serine/Threonine Kinase B	0.008	1.67
KIF26B	Kinesin Family Member 26B	0.020	1.66
SSC4D	Scavenger Receptor Cysteine Rich Family Member With 4 Domains	0.044	1.66
CAV2	Caveolin 2	0.020	1.65
RNF43	Ring Finger Protein 43	0.026	1.64
TLDC2	TBC/Lysm-Associated Domain Containing 2	0.047	1.64
CD80	CD80 Molecule	0.015	1.64
DERL3	Derlin 3	0.010	1.64
KNL1	Kinetochore Scaffold 1	0.032	1.63
SAPCD1	Suppressor APC Domain Containing 1	0.026	1.63
PIF1	PIF1 5'-To-3' DNA Helicase	0.002	1.63
LGALS4	Galectin 4	0.030	1.62
NUAK1	NUAK Family Kinase 1	0.016	1.61
HASPIN	Histone H3 Associated Protein Kinase	0.005	1.61
CKAP2L	Cytoskeleton Associated Protein 2 Like	0.044	1.60
TBC1D3	TBC1 Domain Family Member 3	0.035	1.59
LCN2	Lipocalin 2	0.045	1.59

CDC6	Cell Division Cycle 6	0.025	1.59
CDK1	Cyclin Dependent Kinase 1	0.039	1.58
BMP8B	Bone Morphogenetic Protein 8b	0.005	1.58
SMIM10	Small Integral Membrane Protein 10	0.046	1.57
FSCN2	Fascin Actin-Bundling Protein 2, Retinal	0.009	1.57
ABCA13	ATP Binding Cassette Subfamily A Member 13	0.045	1.57
KIF24	Kinesin Family Member 24	0.001	1.56
CEP128	Centrosomal Protein 128	< 0.001	1.56
BTN1A1	Butyrophilin Subfamily 1 Member A1	0.039	1.56
GPLD1	Glycosylphosphatidylinositol Specific Phospholipase D1	0.010	1.55
CHIT1	Chitinase 1	0.017	1.55
CLSPN	Claspin	0.036	1.54
ARHGAP23	Rho Gtpase Activating Protein 23	0.039	1.53
KIF14	Kinesin Family Member 14	0.037	1.53
GINS2	GINS Complex Subunit 2	0.009	1.52
NRG1	Neuregulin 1	0.013	1.52
TRIM74	Tripartite Motif Containing 74	0.037	1.52
COL4A4	Collagen Type IV Alpha 4 Chain	0.022	1.52
PARM1	Prostate Androgen-Regulated Mucin-Like Protein 1	0.001	1.52
AZU1	Azurocidin 1	0.043	1.52
SSPN	Sarcospan	0.010	1.52
NUGGC	Nuclear Gtpase, Germinal Center Associated	< 0.001	1.51
ALDH1A2	Aldehyde Dehydrogenase 1 Family Member A2	0.028	1.51
CENPM	Centromere Protein M	0.009	1.51
ASPM	Assembly Factor For Spindle Microtubules	0.049	1.51
HP	Haptoglobin	0.029	1.50
ARHGAP42	Rho Gtpase Activating Protein 42	0.044	1.50
CENPE	Centromere Protein E	0.008	1.49
ZNF714	Zinc Finger Protein 714	0.008	1.49
KCND3	Potassium Voltage-Gated Channel Subfamily D Member 3	0.026	1.48

ZWINT	ZW10 Interacting Kinetochore Protein	0.014	1.48
RNF112	Ring Finger Protein 112	0.028	1.48
RETN	Resistin	0.016	1.47
KIFC1	Kinesin Family Member C1	0.021	1.47
NOXRED1	NADP Dependent Oxidoreductase Domain Containing 1	0.013	1.47
AURKB	Aurora Kinase B	0.013	1.45
ARRDC4	Arrestin Domain Containing 4	0.001	1.45
TRIM36	Tripartite Motif Containing 36	0.020	1.45
PHYHD1	Phytanoyl-Coa Dioxygenase Domain Containing 1	0.024	1.45
ELL2	Elongation Factor For RNA Polymerase II 2	0.002	1.44
CCNA2	Cyclin A2	0.021	1.44
GGH	Gamma-Glutamyl Hydrolase	0.008	1.44
SLC1A4	Solute Carrier Family 1 Member 4	0.001	1.43
RIBC1	RIB43A Domain With Coiled-Coils 1	0.024	1.43
CD38	CD38 Molecule	0.020	1.42
CD209	CD209 Molecule	0.029	1.42
LGALSL	Galectin Like	0.011	1.40
MPO	Myeloperoxidase	0.016	1.39
CHRNE	Cholinergic Receptor Nicotinic Epsilon Subunit	0.001	1.39
GTSE1	G2 And S-Phase Expressed 1	0.019	1.39
RGS16	Regulator Of G Protein Signaling 16	0.031	1.39
MYO1D	Myosin ID	0.004	1.39
NT5DC2	5'-Nucleotidase Domain Containing 2	0.037	1.39
CHPF	Chondroitin Polymerizing Factor	0.003	1.38
DENND5B	DENN Domain Containing 5B	0.010	1.38
PERP	P53 Apoptosis Effector Related To PMP22	0.014	1.38
B4GALT6	Beta-1,4-Galactosyltransferase 6	0.046	1.38
UBE2C	Ubiquitin Conjugating Enzyme E2 C	0.015	1.38
PLK1	Polo Like Kinase 1	0.005	1.38
CDCA3	Cell Division Cycle Associated 3	0.024	1.38

DSC2	Desmocollin 2	0.049	1.37
UHRF1	Ubiquitin Like With PHD And Ring Finger Domains 1	0.013	1.37
CAPS2	Calcyphosine 2	0.033	1.37
IRF4	Interferon Regulatory Factor 4	0.004	1.36
PPFIA3	PTPRF Interacting Protein Alpha 3	0.009	1.36
RIPOR3	RIPOR Family Member 3	0.046	1.36
AFDN	Afadin, Adherens Junction Formation Factor	0.027	1.36
TPX2	TPX2 Microtubule Nucleation Factor	0.044	1.36
PKMYT1	Protein Kinase, Membrane Associated Tyrosine/Threonine 1	0.022	1.35
TNFRSF13B	TNF Receptor Superfamily Member 13B	0.035	1.34
EIF5A2	Eukaryotic Translation Initiation Factor 5A2	0.019	1.34
TENT5C	Terminal Nucleotidyltransferase 5C	0.006	1.34
CCNB1	Cyclin B1	0.021	1.33
KIAA0319	Kiaa0319	0.015	1.32
SLC4A5	Solute Carrier Family 4 Member 5	0.009	1.32
DST	Dystonin	0.012	1.31
ANKRD36	Ankyrin Repeat Domain 36	0.028	1.31
FAM174B	Family With Sequence Similarity 174 Member B	0.038	1.31
KLF5	Kruppel Like Factor 5	0.023	1.30
MAP3K7CL	MAP3K7 C-Terminal Like	0.005	1.30
DAAM1	Dishevelled Associated Activator Of Morphogenesis 1	0.005	1.30
SGPP2	Sphingosine-1-Phosphate Phosphatase 2	0.004	1.30
TTF2	Transcription Termination Factor 2	0.016	1.29
SESTD1	SEC14 And Spectrin Domain Containing 1	0.015	1.29
KIF11	Kinesin Family Member 11	0.043	1.28
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.017	1.28
FANCI	FA Complementation Group I	0.016	1.28
PLTP	Phospholipid Transfer Protein	0.025	1.28

NOTCH2NLA	Notch 2 N-Terminal Like A	0.015	1.27
TMEM231	Transmembrane Protein 231	0.019	1.27
PDIA4	Protein Disulfide Isomerase Family A Member 4	0.002	1.27
E2F2	E2F Transcription Factor 2	0.012	1.27
SLC22A5	Solute Carrier Family 22 Member 5	0.014	1.27
SLC2A5	Solute Carrier Family 2 Member 5	0.050	1.26
PALM2AKAP2	PALM2 And AKAP2 Fusion	0.027	1.26
BMP6	Bone Morphogenetic Protein 6	0.005	1.26
SLC44A1	Solute Carrier Family 44 Member 1	0.002	1.26
FAR2	Fatty Acyl-Coa Reductase 2	0.002	1.26
ANKRD37	Ankyrin Repeat Domain 37	0.029	1.26
ARPIN-AP3S2	ARPIN-AP3S2 Readthrough	0.027	1.25
HSP90B1	Heat Shock Protein 90 Beta Family Member 1	0.002	1.25
SPATA6	Spermatogenesis Associated 6	0.038	1.25
PDPR	Pyruvate Dehydrogenase Phosphatase Regulatory Subunit	0.025	1.24
PLA2G12A	Phospholipase A2 Group XIIA	0.040	1.24
FAAP24	FA Core Complex Associated Protein 24	0.039	1.24
TXLNB	Taxilin Beta	0.039	1.24
CCDC170	Coiled-Coil Domain Containing 170	0.048	1.24
CCDC88A	Coiled-Coil Domain Containing 88A	0.007	1.24
MCUR1	Mitochondrial Calcium Uniporter Regulator 1	0.012	1.24
JAG1	Jagged Canonical Notch Ligand 1	0.040	1.24
B3GNT5	UDP-Glcnac:Betagal Beta-1,3-N-Acetylglucosaminyltransferase 5	0.017	1.23
PRIMPOL	Primase And DNA Directed Polymerase	0.018	1.22
ZFHX3	Zinc Finger Homeobox 3	0.006	1.22
REEP3	Receptor Accessory Protein 3	0.005	1.22
GCNT1	Glucosaminyl (N-Acetyl) Transferase 1	0.033	1.22

ZNF324B	Zinc Finger Protein 324B	0.012	1.21
SEC22A	SEC22 Homolog A, Vesicle Trafficking Protein	0.021	1.21
EOGT	EGF Domain Specific O-Linked N-Acetylglucosamine Transferase	0.049	1.21
PALLD	Palladin, Cytoskeletal Associated Protein	0.032	1.21
PLAGL1	PLAG1 Like Zinc Finger 1	0.018	1.21
BRCA1	BRCA1 DNA Repair Associated	0.010	1.21
LARS2	Leucyl-Trna Synthetase 2, Mitochondrial	0.013	1.20
TFR2	Transferrin Receptor 2	0.041	1.20
ZNF501	Zinc Finger Protein 501	0.032	1.20
TMEM128	Transmembrane Protein 128	0.003	-1.20
GPRASP2	G Protein-Coupled Receptor Associated Sorting Protein 2	0.038	-1.20
SHLD1	Shieldin Complex Subunit 1	0.013	-1.21
CARMIL1	Capping Protein Regulator And Myosin 1 Linker 1	0.038	-1.21
NBPF1	NBPF Member 1	0.040	-1.21
PTPRS	Protein Tyrosine Phosphatase Receptor Type S	0.047	-1.22
CD1A	CD1a Molecule	0.045	-1.22
CEP83	Centrosomal Protein 83	0.042	-1.22
ATG9B	Autophagy Related 9B	0.035	-1.22
TNK1	Tyrosine Kinase Non Receptor 1	0.046	-1.22
PNPLA4	Patatin Like Phospholipase Domain Containing 4	0.022	-1.22
OLFM2	Olfactomedin 2	0.013	-1.23
DBN1	Drebrin 1	0.007	-1.23
COQ3	Coenzyme Q3, Methyltransferase	0.023	-1.23
GRAPL	GRB2 Related Adaptor Protein Like	0.037	-1.24
CLEC11A	C-Type Lectin Domain Containing 11A	0.018	-1.24
TTC24	Tetratricopeptide Repeat Domain 24	0.036	-1.24
FAAH	Fatty Acid Amide Hydrolase	0.041	-1.24
WDR27	WD Repeat Domain 27	0.036	-1.25
SPRED1	Sprouty Related EVH1 Domain Containing 1	0.031	-1.25

FBLN2	Fibulin 2	0.043	-1.25
C3orf33	Chromosome 3 Open Reading Frame 33	0.047	-1.26
YEATS4	YEATS Domain Containing 4	0.015	-1.26
PCGF5	Polycomb Group Ring Finger 5	0.049	-1.26
DLG5	Discs Large MAGUK Scaffold Protein 5	0.045	-1.27
PKIA	Camp-Dependent Protein Kinase Inhibitor Alpha	0.010	-1.27
CD248	CD248 Molecule	0.020	-1.28
FGGY	FGGY Carbohydrate Kinase Domain Containing	0.015	-1.28
IMMP2L	Inner Mitochondrial Membrane Peptidase Subunit 2	0.037	-1.29
RPS28	Ribosomal Protein S28	0.017	-1.31
CACHD1	Cache Domain Containing 1	0.019	-1.31
ST8SIA1	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 1	0.029	-1.32
ASAH2B	N-Acylsphingosine Amidohydrolase 2B	0.020	-1.32
MAD1L1	Mitotic Arrest Deficient 1 Like 1	0.003	-1.32
HBA1	Hemoglobin Subunit Alpha 1	0.047	-1.32
WHRN	Whirlin	0.036	-1.32
MMEL1	Membrane Metalloendopeptidase Like 1	0.028	-1.32
SUSD4	Sushi Domain Containing 4	0.038	-1.32
WASF1	WASP Family Member 1	0.005	-1.33
EFCAB7	EF-Hand Calcium Binding Domain 7	0.015	-1.33
ZRANB3	Zinc Finger RANBP2-Type Containing 3	0.044	-1.33
PLVAP	Plasmalemma Vesicle Associated Protein	0.042	-1.33
SIRT4	Sirtuin 4	0.026	-1.34
PRTFDC1	Phosphoribosyl Transferase Domain Containing 1	0.048	-1.37
PDE9A	Phosphodiesterase 9A	0.012	-1.37
FAM184A	Family With Sequence Similarity 184 Member A	0.042	-1.37
CNN3	Calponin 3	0.004	-1.38
CLEC4C	C-Type Lectin Domain Family 4 Member C	0.041	-1.39
PTGES	Prostaglandin E Synthase	0.040	-1.39
NRIP3	Nuclear Receptor Interacting Protein 3	0.015	-1.42

TKTL1	Transketolase Like 1	0.009	-1.43
CUX2	Cut Like Homeobox 2	0.016	-1.43
KHDRBS2	KH RNA Binding Domain Containing, Signal Transduction Associated 2	0.014	-1.43
SOX8	SRY-Box Transcription Factor 8	0.011	-1.43
ANK2	Ankyrin 2	0.044	-1.43
NRCAM	Neuronal Cell Adhesion Molecule	0.021	-1.43
RBFOX2	RNA Binding Fox-1 Homolog 2	0.041	-1.46
PPP2R3A	Protein Phosphatase 2 Regulatory Subunit B''alpha	0.035	-1.46
ZNF835	Zinc Finger Protein 835	0.014	-1.46
GRIK5	Glutamate Ionotropic Receptor Kainate Type Subunit 5	0.036	-1.46
ZNF177	Zinc Finger Protein 177	0.021	-1.46
MYH10	Myosin Heavy Chain 10	0.033	-1.47
SLC16A11	Solute Carrier Family 16 Member 11	0.015	-1.47
ZDHHC11B	Zinc Finger DHHC-Type Containing 11B	0.036	-1.47
GARNL3	Gtpase Activating Rap/Rangap Domain Like 3	0.022	-1.48
GCAT	Glycine C-Acetyltransferase	0.013	-1.50
TTC39A	Tetratricopeptide Repeat Domain 39A	0.028	-1.50
NRXN2	Neurexin 2	0.015	-1.51
KIF5A	Kinesin Family Member 5A	0.036	-1.53
STARD13	Star Related Lipid Transfer Domain Containing 13	0.040	-1.53
ATP1A2	Atpase Na+/K+ Transporting Subunit Alpha 2	0.042	-1.54
ABCA6	ATP Binding Cassette Subfamily A Member 6	0.046	-1.56
LMCD1	LIM And Cysteine Rich Domains 1	0.017	-1.56
GYPE	Glycophorin E (MNS Blood Group)	0.039	-1.56
KIRREL3	Kirre Like Nephrin Family Adhesion Molecule 3	0.044	-1.57
SDC2	Syndecan 2	0.036	-1.57
SPTSSB	Serine Palmitoyltransferase Small Subunit B	0.034	-1.57
STXBP4	Syntaxin Binding Protein 4	0.003	-1.58
MYO1A	Myosin IA	0.023	-1.58

H4C4	H4 Clustered Histone 4	0.031	-1.59
IFITM3	Interferon Induced Transmembrane Protein 3	0.036	-1.60
PDZD7	PDZ Domain Containing 7	0.016	-1.62
GRM7	Glutamate Metabotropic Receptor 7	0.030	-1.63
SFRP5	Secreted Frizzled Related Protein 5	0.031	-1.64
GPD1	Glycerol-3-Phosphate Dehydrogenase 1	0.021	-1.64
APBA1	Amyloid Beta Precursor Protein Binding Family A Member 1	0.001	-1.65
ASB9	Ankyrin Repeat And SOCS Box Containing 9	0.046	-1.66
EPB41L1	Erythrocyte Membrane Protein Band 4.1 Like 1	0.009	-1.66
TG	Thyroglobulin	0.017	-1.67
TLN2	Talin 2	0.021	-1.69
ELF3	E74 Like ETS Transcription Factor 3	0.040	-1.69
CCDC171	Coiled-Coil Domain Containing 171	0.004	-1.70
CTNNA2	Catenin Alpha 2	0.038	-1.70
TTLL10	Tubulin Tyrosine Ligase Like 10	0.026	-1.72
C17orf97	Chromosome 17 Open Reading Frame 97	0.027	-1.72
TUBB2A	Tubulin Beta 2A Class Iia	0.024	-1.73
CTRC	Chymotrypsin C	0.015	-1.74
FEZF2	FEZ Family Zinc Finger 2	0.019	-1.74
FOLR3	Folate Receptor Gamma	0.034	-1.74
TNR	Tenascin R	0.048	-1.75
TACSTD2	Tumor Associated Calcium Signal Transducer 2	0.044	-1.77
CERS3	Ceramide Synthase 3	0.036	-1.78
PRR36	Proline Rich 36	0.013	-1.82
DSG2	Desmoglein 2	0.040	-1.82
LPL	Lipoprotein Lipase	0.012	-1.82
NOTCH3	Notch Receptor 3	0.010	-1.83
MXRA8	Matrix Remodeling Associated 8	0.001	-1.84
MAGI1	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 1	0.021	-1.86

FNDC5	Fibronectin Type III Domain Containing 5	0.010	-1.86
RALYL	RALY RNA Binding Protein Like	0.034	-1.91
SLC7A9	Solute Carrier Family 7 Member 9	0.004	-1.92
PPIAL4E	Peptidylprolyl Isomerase A Like 4E	0.031	-1.93
FOXH1	Forkhead Box H1	0.034	-1.96
SIGLEC11	Sialic Acid Binding Ig Like Lectin 11	0.001	-1.99
INSYN2B	Inhibitory Synaptic Factor Family Member 2B	0.009	-2.07
GSTA1	Glutathione S-Transferase Alpha 1	0.023	-2.10
MAGI2	Membrane Associated Guanylate Kinase, WW And PDZ Domain Containing 2	0.014	-2.11
PLS3	Plastin 3	< 0.001	-2.11
COLEC12	Collectin Subfamily Member 12	0.001	-2.13
LRRC36	Leucine Rich Repeat Containing 36	< 0.001	-2.15
ROBO1	Roundabout Guidance Receptor 1	0.003	-2.18
PRKD1	Protein Kinase D1	0.046	-2.29
EIF2S3B	Eukaryotic Translation Initiation Factor 2 Subunit Gamma B	0.007	-2.32
MEDAG	Mesenteric Estrogen Dependent Adipogenesis	0.045	-2.35
HBZ	Hemoglobin Subunit Zeta	0.019	-2.39
ST18	ST18 C2H2C-Type Zinc Finger Transcription Factor	0.023	-2.44
CDO1	Cysteine Dioxygenase Type 1	0.028	-2.48
CFAP46	Cilia And Flagella Associated Protein 46	0.016	-2.49
PNMA8B	PNMA Family Member 8B	0.001	-2.64
GLP1R	Glucagon Like Peptide 1 Receptor	0.009	-2.66
KCNJ10	Potassium Inwardly Rectifying Channel Subfamily J Member 10	< 0.001	-2.81
KCNQ2	Potassium Voltage-Gated Channel Subfamily Q Member 2	0.005	-2.96
SLC6A20	Solute Carrier Family 6 Member 20	0.002	-3.01
DPP6	Dipeptidyl Peptidase Like 6	0.050	-3.02
INSYN2A	Inhibitory Synaptic Factor 2A	0.003	-3.10
DCHS2	Dachsous Cadherin-Related 2	0.016	-3.12
CBLN2	Cerebellin 2 Precursor	0.003	-3.13

TREML4	Triggering Receptor Expressed On Myeloid Cells Like 4	0.043	-4.97
CTAGE8	CTAGE Family Member 8	0.004	-7.13
CYP26B1	Cytochrome P450 Family 26 Subfamily B Member 1	< 0.001	-21.32

Table 6E. List of 359 genes differently modulated in males HR vs males LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
PCDHA6	Protocadherin Alpha 6	0.037	90.93
TBC1D3K	TBC1 Domain Family Member 3K	0.047	71.15
TBC1D3	TBC1 Domain Family Member 3	0.016	14.00
DLGAP2	DLG Associated Protein 2	0.001	10.36
MGAT4C	MGAT4 Family Member C	0.015	4.52
AS3MT	Arsenite Methyltransferase	0.012	4.20
SLC12A1	Solute Carrier Family 12 Member 1	0.017	3.25
TRIM72	Tripartite Motif Containing 72	< 0.001	3.17
CCDC110	Coiled-Coil Domain Containing 110	0.016	3.12
KLHL41	Kelch Like Family Member 41	0.032	2.98
CA12	Carbonic Anhydrase 12	0.007	2.96
DCC	DCC Netrin 1 Receptor	0.036	2.88
AGT	Angiotensinogen	0.002	2.80
SULT1A4	Sulfotransferase Family 1A Member 4	< 0.001	2.79
PROX1	Prospero Homeobox 1	0.034	2.68
VWA5B2	Von Willebrand Factor A Domain Containing 5B2	0.035	2.67
EVA1A	Eva-1 Homolog A, Regulator Of Programmed Cell Death	0.043	2.67
PPL	Periplakin	0.002	2.64
KCND2	Potassium Voltage-Gated Channel Subfamily D Member 2	0.021	2.63
ESRP2	Epithelial Splicing Regulatory Protein 2	0.018	2.63
FAM184B	Family With Sequence Similarity 184 Member B	0.001	2.62
SYCE1	Synaptonemal Complex Central Element Protein 1	0.043	2.55
CHRNA4	Cholinergic Receptor Nicotinic Alpha 4 Subunit	0.025	2.55
TMEM132B	Transmembrane Protein 132B	0.028	2.54
NR2F2	Nuclear Receptor Subfamily 2 Group F Member 2	0.022	2.51

KIAA1614	Kiaa1614	0.010	2.46
ASCL5	Achaete-Scute Family Bhlh Transcription Factor 5	0.027	2.36
CERKL	Ceramide Kinase Like	0.043	2.25
KCNQ2	Potassium Voltage-Gated Channel Subfamily Q Member 2	0.041	2.25
ZNF492	Zinc Finger Protein 492	0.008	2.23
IQGAP3	IQ Motif Containing Gtpase Activating Protein 3	0.001	2.20
LGI3	Leucine Rich Repeat LGI Family Member 3	0.045	2.20
HPSE2	Heparanase 2 (Inactive)	0.028	2.18
ATP6V1C2	Atpase H+ Transporting V1 Subunit C2	0.016	2.17
TMEM200B	Transmembrane Protein 200B	0.009	2.17
B3GAT2	Beta-1,3-Glucuronyltransferase 2	0.027	2.16
SCN5A	Sodium Voltage-Gated Channel Alpha Subunit 5	0.042	2.13
SLC6A13	Solute Carrier Family 6 Member 13	0.004	2.12
CAVIN4	Caveolae Associated Protein 4	0.019	2.05
SPATA21	Spermatogenesis Associated 21	0.012	1.98
RGS5	Regulator Of G Protein Signaling 5	0.043	1.94
C17orf97	Chromosome 17 Open Reading Frame 97	0.015	1.91
C6orf52	Chromosome 6 Open Reading Frame 52	0.038	1.89
CHRNA5	Cholinergic Receptor Nicotinic Alpha 5 Subunit	0.017	1.87
LONRF2	LON Peptidase N-Terminal Domain And Ring Finger 2	0.043	1.86
HSD17B14	Hydroxysteroid 17-Beta Dehydrogenase 14	0.026	1.86
SLC26A1	Solute Carrier Family 26 Member 1	0.006	1.83
DOC2B	Double C2 Domain Beta	0.024	1.83
SH3RF2	SH3 Domain Containing Ring Finger 2	0.035	1.75
STKLD1	Serine/Threonine Kinase Like Domain Containing 1	0.030	1.75
AMH	Anti-Mullerian Hormone	0.008	1.74
CHD5	Chromodomain Helicase DNA Binding Protein 5	0.045	1.73
TMEM191B	Transmembrane Protein 191B	0.026	1.72

PLEKHA6	Pleckstrin Homology Domain Containing A6	0.011	1.70
CFAP73	Cilia And Flagella Associated Protein 73	0.045	1.70
TLDC2	TBC/Lysm-Associated Domain Containing 2	0.047	1.70
LAIR2	Leukocyte Associated Immunoglobulin Like Receptor 2	0.035	1.70
EHF	ETS Homologous Factor	0.021	1.69
TMEM262	Transmembrane Protein 262	0.031	1.67
WDR17	WD Repeat Domain 17	0.013	1.67
ALDH1A2	Aldehyde Dehydrogenase 1 Family Member A2	0.004	1.67
CTRC	Chymotrypsin C	0.014	1.66
EREG	Epiregulin	0.039	1.65
CYP21A2	Cytochrome P450 Family 21 Subfamily A Member 2	0.031	1.64
ZACN	Zinc Activated Ion Channel	0.002	1.64
GOLGA6L4	Golgin A6 Family Like 4	0.030	1.64
SERPINB10	Serpin Family B Member 10	0.048	1.62
AJUBA	Ajuba LIM Protein	0.005	1.61
PTTG2	Pituitary Tumor-Transforming 2	0.006	1.59
SHISA7	Shisa Family Member 7	0.029	1.59
KCNK17	Potassium Two Pore Domain Channel Subfamily K Member 17	0.044	1.59
DCLK2	Doublecortin Like Kinase 2	0.017	1.58
DCBLD2	Discoidin, CUB And LCCL Domain Containing 2	0.005	1.56
MYRF	Myelin Regulatory Factor	0.022	1.55
RAPGEF3	Rap Guanine Nucleotide Exchange Factor 3	0.021	1.54
SNX32	Sorting Nexin 32	0.009	1.54
TMEM198	Transmembrane Protein 198	0.021	1.54
TLN2	Talin 2	0.029	1.53
CFAP97D2	CFAP97 Domain Containing 2	0.019	1.53
IL12A	Interleukin 12A	0.012	1.53
CXCL8	C-X-C Motif Chemokine Ligand 8	0.013	1.52
NKD1	NKD Inhibitor Of WNT Signaling Pathway 1	0.007	1.50
EML6	EMAP Like 6	0.043	1.49

ZNF714	Zinc Finger Protein 714	0.005	1.49
VNN1	Vanin 1	0.007	1.48
PIF1	PIF1 5'-To-3' DNA Helicase	0.013	1.48
KLRC1	Killer Cell Lectin Like Receptor C1	0.005	1.48
MMP24	Matrix Metallopeptidase 24	0.048	1.47
IRAK1BP1	Interleukin 1 Receptor Associated Kinase 1 Binding Protein 1	0.014	1.43
FBXO36	F-Box Protein 36	0.006	1.43
LAMB2	Laminin Subunit Beta 2	0.048	1.43
GPLD1	Glycosylphosphatidylinositol Specific Phospholipase D1	0.024	1.43
TRPC1	Transient Receptor Potential Cation Channel Subfamily C Member 1	0.020	1.42
STARD4	Star Related Lipid Transfer Domain Containing 4	0.014	1.42
GINS2	GINS Complex Subunit 2	0.023	1.42
LRRC69	Leucine Rich Repeat Containing 69	0.043	1.40
MTCL1	Microtubule Crosslinking Factor 1	0.045	1.40
ELAPOR2	Endosome-Lysosome Associated Apoptosis And Autophagy Regulator Family Member 2	0.021	1.39
TTC23	Tetratricopeptide Repeat Domain 23	0.050	1.38
REELD1	Reeler Domain Containing 1	0.041	1.38
MANEA	Mannosidase Endo-Alpha	0.015	1.37
GOLGA8H	Golgin A8 Family Member H	0.014	1.37
ZBTB41	Zinc Finger And BTB Domain Containing 41	0.047	1.37
PTGDS	Prostaglandin D2 Synthase	0.014	1.37
KIAA0895L	KIAA0895 Like	0.004	1.36
SLC4A10	Solute Carrier Family 4 Member 10	0.042	1.36
ARMCX4	Armadillo Repeat Containing X-Linked 4	0.007	1.35
BRMS1L	BRMS1 Like Transcriptional Repressor	0.009	1.35
IKZF2	IKAROS Family Zinc Finger 2	0.027	1.34
LOC102724488	Synaptotagmin-15	0.045	1.34
RAD51AP1	RAD51 Associated Protein 1	0.045	1.34

ENPP4	Ectonucleotide Pyrophosphatase/Phosphodiesterase 4	0.016	1.33
OPHN1	Oligophrenin 1	0.010	1.33
CCR9	C-C Motif Chemokine Receptor 9	0.040	1.33
HSF4	Heat Shock Transcription Factor 4	0.017	1.33
LTK	Leukocyte Receptor Tyrosine Kinase	0.026	1.33
SYNGR1	Synaptogyrin 1	0.008	1.33
ZNF850	Zinc Finger Protein 850	0.022	1.33
CLDN12	Claudin 12	0.032	1.32
DENND1B	DENN Domain Containing 1B	0.013	1.32
ZNF600	Zinc Finger Protein 600	0.005	1.32
SPTBN5	Spectrin Beta, Non-Erythrocytic 5	0.023	1.32
PIWIL4	Piwi Like RNA-Mediated Gene Silencing 4	0.026	1.32
TMEM237	Transmembrane Protein 237	0.009	1.32
TENM1	Teneurin Transmembrane Protein 1	0.023	1.31
DUSP19	Dual Specificity Phosphatase 19	0.045	1.31
TMEM67	Transmembrane Protein 67	0.044	1.31
SLC19A2	Solute Carrier Family 19 Member 2	0.012	1.31
SNX16	Sorting Nexin 16	0.016	1.31
FAM169A	Family With Sequence Similarity 169 Member A	0.048	1.30
FZD6	Frizzled Class Receptor 6	0.032	1.30
IL15	Interleukin 15	0.045	1.30
TMEM144	Transmembrane Protein 144	0.026	1.30
RAVER2	Ribonucleoprotein, PTB Binding 2	0.004	1.30
HKDC1	Hexokinase Domain Containing 1	0.048	1.29
ZNF205	Zinc Finger Protein 205	0.024	1.29
ADAMTS10	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 10	0.021	1.29
OR4D1	Olfactory Receptor Family 4 Subfamily D Member 1	0.026	1.29
ND6	NADH Dehydrogenase Subunit 6	0.049	1.29
SLC4A4	Solute Carrier Family 4 Member 4	0.020	1.29

LRRN1	Leucine Rich Repeat Neuronal 1	0.039	1.28
RAB40B	RAB40B, Member RAS Oncogene Family	0.049	1.28
TMX3	Thioredoxin Related Transmembrane Protein 3	0.008	1.28
OSBPL8	Oxysterol Binding Protein Like 8	0.011	1.28
ARVCF	ARVCF Delta Catenin Family Member	0.028	1.28
RCN3	Reticulocalbin 3	0.020	1.28
MAP3K21	Mitogen-Activated Protein Kinase Kinase Kinase 21	0.020	1.28
FAM221A	Family With Sequence Similarity 221 Member A	0.042	1.28
KBTBD8	Kelch Repeat And BTB Domain Containing 8	0.036	1.28
PRKCI	Protein Kinase C Iota	0.007	1.28
ITGAV	Integrin Subunit Alpha V	0.015	1.28
GCNT4	Glucosaminyl (N-Acetyl) Transferase 4	0.041	1.28
MEX3B	Mex-3 RNA Binding Family Member B	0.047	1.27
HSPA13	Heat Shock Protein Family A (Hsp70) Member 13	0.009	1.27
HES6	Hes Family Bhlh Transcription Factor 6	0.010	1.27
RAP2A	RAP2A, Member Of RAS Oncogene Family	0.018	1.27
IGSF9B	Immunoglobulin Superfamily Member 9B	0.046	1.27
ARHGAP5	Rho Gtpase Activating Protein 5	0.011	1.27
CPEB2	Cytoplasmic Polyadenylation Element Binding Protein 2	0.019	1.26
TGFBR3	Transforming Growth Factor Beta Receptor 3	0.026	1.26
TOGARAM 1	TOG Array Regulator Of Axonemal Microtubules 1	0.035	1.26
CD151	CD151 Molecule (Raph Blood Group)	0.030	1.26
COL6A2	Collagen Type VI Alpha 2 Chain	0.019	1.26
CCDC30	Coiled-Coil Domain Containing 30	0.024	1.26
LRRC24	Leucine Rich Repeat Containing 24	0.016	1.26
DGKQ	Diacylglycerol Kinase Theta	0.016	1.26
CCDC14	Coiled-Coil Domain Containing 14	0.005	1.26
TET1	Tet Methylcytosine Dioxygenase 1	0.040	1.26
SLC30A4	Solute Carrier Family 30 Member 4	0.014	1.25

ECT2	Epithelial Cell Transforming 2	0.032	1.25
P2RX1	Purinergic Receptor P2X 1	0.026	1.25
VPS13A	Vacuolar Protein Sorting 13 Homolog A	0.025	1.25
ZDHHC17	Zinc Finger DHHC-Type Palmitoyltransferase 17	0.019	1.25
RAB11FIP2	RAB11 Family Interacting Protein 2	0.018	1.25
UCN	Urocortin	0.050	1.25
USP45	Ubiquitin Specific Peptidase 45	0.021	1.25
FOXN2	Forkhead Box N2	0.010	1.24
FIGNL2	Fidgetin Like 2	0.047	1.24
KLHL17	Kelch Like Family Member 17	0.033	1.24
MYSM1	Myb Like, SWIRM And MPN Domains 1	0.022	1.23
MIER3	MIER Family Member 3	0.021	1.23
CARNMT1	Carnosine N-Methyltransferase 1	0.021	1.23
SLC4A7	Solute Carrier Family 4 Member 7	0.046	1.23
SEMA6C	Semaphorin 6C	0.025	1.23
OSBPL5	Oxysterol Binding Protein Like 5	0.010	1.23
PI4K2B	Phosphatidylinositol 4-Kinase Type 2 Beta	0.049	1.23
ETNK1	Ethanolamine Kinase 1	0.020	1.23
SHPRH	SNF2 Histone Linker PHD RING Helicase	0.004	1.23
MIB1	MIB E3 Ubiquitin Protein Ligase 1	0.042	1.23
NMRK1	Nicotinamide Riboside Kinase 1	0.005	1.22
PRIMPOL	Primase And DNA Directed Polymerase	0.027	1.22
ZC3H6	Zinc Finger CCCH-Type Containing 6	0.008	1.22
KCNH2	Potassium Voltage-Gated Channel Subfamily H Member 2	0.042	1.22
PDIK1L	PDLIM1 Interacting Kinase 1 Like	0.009	1.22
TMTC4	Transmembrane O-Mannosyltransferase Targeting Cadherins 4	0.031	1.22
EPHA4	EPH Receptor A4	0.043	1.22
ADCK5	Aarf Domain Containing Kinase 5	0.006	1.22
TP53I13	Tumor Protein P53 Inducible Protein 13	0.005	1.22
VAMP1	Vesicle Associated Membrane Protein 1	0.004	1.22

FASTKD1	FAST Kinase Domains 1	0.036	1.22
SLC25A29	Solute Carrier Family 25 Member 29	0.044	1.21
ZNF331	Zinc Finger Protein 331	0.001	1.21
ENGASE	Endo-Beta-N-Acetylglucosaminidase	0.035	1.21
CFAP418	Cilia And Flagella Associated Protein 418	0.049	1.21
CEP192	Centrosomal Protein 192	0.003	1.21
PKN2	Protein Kinase N2	0.018	1.21
GMFB	Glia Maturation Factor Beta	0.041	1.21
PACRGL	Parkin Coregulated Like	0.031	1.21
TRUB1	Trub Pseudouridine Synthase Family Member 1	0.029	1.21
NAA16	N-Alpha-Acetyltransferase 16, Nata Auxiliary Subunit	0.009	1.21
TCF19	Transcription Factor 19	0.006	1.21
PGM2L1	Phosphoglucomutase 2 Like 1	0.021	1.20
CROT	Carnitine O-Octanoyltransferase	0.034	1.20
ZNF599	Zinc Finger Protein 599	0.020	1.20
SMIM13	Small Integral Membrane Protein 13	0.025	1.20
UFC1	Ubiquitin-Fold Modifier Conjugating Enzyme 1	0.039	-1.20
ATP5PB	ATP Synthase Peripheral Stalk-Membrane Subunit B	0.048	-1.20
SCAPER	S-Phase Cyclin A Associated Protein In The ER	0.038	-1.20
AIMP1	Aminoacyl Trna Synthetase Complex Interacting Multifunctional Protein 1	0.016	-1.21
WDPCP	WD Repeat Containing Planar Cell Polarity Effector	0.010	-1.21
MTHFD1L	Methylenetetrahydrofolate Dehydrogenase (NADP+ Dependent) 1 Like	0.035	-1.21
ZBTB8OS	Zinc Finger And BTB Domain Containing 8 Opposite Strand	0.014	-1.21
UST	Uronyl 2-Sulfotransferase	0.036	-1.21
MTIF3	Mitochondrial Translational Initiation Factor 3	0.014	-1.21
RPAP3	RNA Polymerase II Associated Protein 3	0.035	-1.22
C2orf81	Chromosome 2 Open Reading Frame 81	0.043	-1.22
THYN1	Thymocyte Nuclear Protein 1	0.005	-1.22

GAS8	Growth Arrest Specific 8	0.033	-1.22
UPF3B	UPF3B Regulator Of Nonsense Mediated Mrna Decay	0.021	-1.22
GEMIN6	Gem Nuclear Organelle Associated Protein 6	0.024	-1.22
TMSB10	Thymosin Beta 10	0.036	-1.23
THG1L	Trna-Histidine Guanylyltransferase 1 Like	0.008	-1.23
PTK2	Protein Tyrosine Kinase 2	0.002	-1.23
RBM8A	RNA Binding Motif Protein 8A	0.024	-1.23
FCER1G	Fc Fragment Of Ige Receptor Ig	0.034	-1.23
CWC15	CWC15 Spliceosome Associated Protein Homolog	0.038	-1.23
PRICKLE1	Prickle Planar Cell Polarity Protein 1	0.037	-1.24
ZNF565	Zinc Finger Protein 565	0.007	-1.24
TIPIN	TIMELESS Interacting Protein	0.035	-1.24
PHAX	Phosphorylated Adaptor For RNA Export	0.044	-1.24
COX17	Cytochrome C Oxidase Copper Chaperone COX17	0.017	-1.25
HEATR5A	HEAT Repeat Containing 5A	0.011	-1.25
SNX8	Sorting Nexin 8	0.028	-1.25
NUTM2E	NUT Family Member 2E	0.039	-1.25
LLPH	LLP Homolog, Long-Term Synaptic Facilitation Factor	0.026	-1.25
NKIRAS1	NFKB Inhibitor Interacting Ras Like 1	0.040	-1.26
RNASE6	Ribonuclease A Family Member K6	0.024	-1.26
VRK1	VRK Serine/Threonine Kinase 1	0.037	-1.27
CCDC170	Coiled-Coil Domain Containing 170	0.028	-1.28
DNM3	Dynamin 3	0.036	-1.28
MRPL39	Mitochondrial Ribosomal Protein L39	0.044	-1.29
MIX23	Mitochondrial Matrix Import Factor 23	0.049	-1.29
CASP1	Caspase 1	0.025	-1.29
LRRC37A2	Leucine Rich Repeat Containing 37 Member A2	0.027	-1.29
LARP7	La Ribonucleoprotein 7, Transcriptional Regulator	0.050	-1.29
CLU	Clusterin	0.044	-1.29
PSMC2	Proteasome 26S Subunit, Atpase 2	0.024	-1.30

PBDC1	Polysaccharide Biosynthesis Domain Containing 1	0.042	-1.30
CWC27	CWC27 Spliceosome Associated Cyclophilin	0.019	-1.31
APOBEC3H	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3H	0.034	-1.31
CIR1	Corepressor Interacting With RBPJ, CIR1	0.013	-1.31
PUM3	Pumilio RNA Binding Family Member 3	< 0.001	-1.32
PFDN2	Prefoldin Subunit 2	0.012	-1.32
PTGR2	Prostaglandin Reductase 2	0.038	-1.33
STAP1	Signal Transducing Adaptor Family Member 1	0.017	-1.33
CFDP1	Craniofacial Development Protein 1	0.038	-1.33
H1-2	H1.2 Linker Histone, Cluster Member	0.031	-1.33
PSD3	Pleckstrin And Sec7 Domain Containing 3	0.042	-1.33
TBCA	Tubulin Folding Cofactor A	0.038	-1.34
TYW1B	Trna-Yw Synthesizing Protein 1 Homolog B	0.040	-1.35
ATP5PO	ATP Synthase Peripheral Stalk Subunit OSCP	0.039	-1.36
GIMAP7	Gtpase, IMAP Family Member 7	0.043	-1.36
ZNF660	Zinc Finger Protein 660	0.050	-1.36
ENY2	ENY2 Transcription And Export Complex 2 Subunit	0.034	-1.37
PPIG	Peptidylprolyl Isomerase G	0.045	-1.38
ATP5PF	ATP Synthase Peripheral Stalk Subunit F6	0.044	-1.38
KANK1	KN Motif And Ankyrin Repeat Domains 1	0.026	-1.39
ZMAT2	Zinc Finger Matrin-Type 2	0.028	-1.40
PSMA3	Proteasome 20S Subunit Alpha 3	0.027	-1.40
TRIM36	Tripartite Motif Containing 36	0.029	-1.40
FCGR1A	Fc Fragment Of Igg Receptor Ia	0.024	-1.40
NINL	Ninein Like	0.018	-1.42
GLB1L	Galactosidase Beta 1 Like	0.005	-1.42
SRP14	Signal Recognition Particle 14	0.033	-1.42
CPXM1	Carboxypeptidase X, M14 Family Member 1	0.049	-1.43
AIF1	Allograft Inflammatory Factor 1	0.023	-1.43
ZNF20	Zinc Finger Protein 20	0.039	-1.43

MRPL51	Mitochondrial Ribosomal Protein L51	0.020	-1.43
RPL36AL	Ribosomal Protein L36a Like	0.007	-1.45
CLEC1B	C-Type Lectin Domain Family 1 Member B	0.020	-1.45
CPA3	Carboxypeptidase A3	0.014	-1.45
IL20RB	Interleukin 20 Receptor Subunit Beta	0.014	-1.46
GGT5	Gamma-Glutamyltransferase 5	0.032	-1.46
C2orf74	Chromosome 2 Open Reading Frame 74	0.016	-1.48
ATP5ME	ATP Synthase Membrane Subunit E	0.036	-1.48
MRPL47	Mitochondrial Ribosomal Protein L47	0.034	-1.49
RPL22	Ribosomal Protein L22	0.042	-1.49
C4A	Complement C4A (Rodgers Blood Group)	0.040	-1.49
RNLS	Renalase, FAD Dependent Amine Oxidase	0.005	-1.49
NKAPL	NFKB Activating Protein Like	0.011	-1.50
C1orf54	Chromosome 1 Open Reading Frame 54	0.011	-1.50
FABP5	Fatty Acid Binding Protein 5	0.013	-1.51
TTC39A	Tetratricopeptide Repeat Domain 39A	0.019	-1.51
MTARC2	Mitochondrial Amidoxime Reducing Component 2	0.016	-1.51
IL31RA	Interleukin 31 Receptor A	0.036	-1.51
AP1M2	Adaptor Related Protein Complex 1 Subunit Mu 2	0.041	-1.52
PIN4	Peptidylprolyl Cis/Trans Isomerase, NIMA-Interacting 4	0.020	-1.52
FAM153B	Family With Sequence Similarity 153 Member B	0.042	-1.54
PLCD3	Phospholipase C Delta 3	0.002	-1.55
MN1	MN1 Proto-Oncogene, Transcriptional Regulator	0.013	-1.56
DBI	Diazepam Binding Inhibitor, Acyl-Coa Binding Protein	0.020	-1.56
NDUFS5	NADH:Ubiquinone Oxidoreductase Subunit S5	0.028	-1.57
PTGR1	Prostaglandin Reductase 1	0.044	-1.58
RPS23	Ribosomal Protein S23	0.020	-1.60
LRRC37A	Leucine Rich Repeat Containing 37A	0.002	-1.63
ALG14	ALG14 UDP-N-Acetylglucosaminyltransferase Subunit	0.017	-1.64
SARNP	SAP Domain Containing Ribonucleoprotein	0.035	-1.64

NDUFA1	NADH:Ubiquinone Oxidoreductase Subunit A1	0.015	-1.65
EMC2	ER Membrane Protein Complex Subunit 2	0.025	-1.66
SNRPD1	Small Nuclear Ribonucleoprotein D1 Polypeptide	0.033	-1.66
NOTCH2NLB	Notch 2 N-Terminal Like B	0.033	-1.66
APOL4	Apolipoprotein L4	0.030	-1.67
KRT72	Keratin 72	0.020	-1.68
MINDY4	MINDY Lysine 48 Deubiquitinase 4	0.030	-1.69
KRT8	Keratin 8	0.009	-1.69
ABCA6	ATP Binding Cassette Subfamily A Member 6	0.013	-1.70
KRT73	Keratin 73	0.008	-1.70
HOXA3	Homeobox A3	0.040	-1.73
CHDH	Choline Dehydrogenase	0.009	-1.76
RPL26	Ribosomal Protein L26	0.044	-1.77
HS3ST1	Heparan Sulfate-Glucosamine 3-Sulfotransferase 1	0.002	-1.78
SEM1	SEM1 26S Proteasome Subunit	0.006	-1.80
DMC1	DNA Meiotic Recombinase 1	0.049	-1.86
B4GALNT3	Beta-1,4-N-Acetyl-Galactosaminyltransferase 3	0.033	-1.87
NECTIN2	Nectin Cell Adhesion Molecule 2	0.020	-1.94
SAMD13	Sterile Alpha Motif Domain Containing 13	0.019	-1.94
CARD16	Caspase Recruitment Domain Family Member 16	0.015	-1.99
CADM2	Cell Adhesion Molecule 2	0.003	-2.11
DUOX2	Dual Oxidase 2	0.030	-2.11
SOX6	SRY-Box Transcription Factor 6	0.005	-2.12
PKIB	Camp-Dependent Protein Kinase Inhibitor Beta	0.003	-2.17
MTUS2	Microtubule Associated Scaffold Protein 2	0.025	-2.19
SOX5	SRY-Box Transcription Factor 5	0.002	-2.22
RPL36A-HNRNPH2	RPL36A-HNRNPH2 Readthrough	0.017	-2.26
RIMBP3C	RIMS Binding Protein 3C	0.039	-2.30

SLC28A2	Solute Carrier Family 28 Member 2	0.030	-2.36
RP1L1	RP1 Like 1	0.036	-2.51
CDH5	Cadherin 5	0.020	-2.52
RPL36A	Ribosomal Protein L36a	0.004	-2.54
UQCRB	Ubiquinol-Cytochrome C Reductase Binding Protein	0.003	-2.57
STAC	SH3 And Cysteine Rich Domain	0.001	-2.66
RGS8	Regulator Of G Protein Signaling 8	0.039	-2.75
FAM81B	Family With Sequence Similarity 81 Member B	0.018	-2.95
RPRM	Reprimo, TP53 Dependent G2 Arrest Mediator Homolog	0.046	-3.17
SNCAIP	Synuclein Alpha Interacting Protein	0.023	-3.21
POU2F3	POU Class 2 Homeobox 3	0.030	-3.56
OR6N1	Olfactory Receptor Family 6 Subfamily N Member 1	0.002	-4.67
CTAGE8	CTAGE Family Member 8	0.007	-6.19
TBC1D3E	TBC1 Domain Family Member 3E	0.009	-122.10

Table 7E. List of 399 genes differently modulated in females MDD vs females HR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05).

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3	TBC1 Domain Family Member 3	0.001	30.28
ADARB2	Adenosine Deaminase RNA Specific B2 (Inactive)	0.005	6.48
NDST3	N-Deacetylase And N-Sulfotransferase 3	0.011	4.21
CCL8	C-C Motif Chemokine Ligand 8	0.005	3.70
KLHL41	Kelch Like Family Member 41	0.006	3.62
GPR26	G Protein-Coupled Receptor 26	0.046	3.32
OR2C3	Olfactory Receptor Family 2 Subfamily C Member 3	0.019	3.01
OR6K3	Olfactory Receptor Family 6 Subfamily K Member 3	0.004	2.92
PLSCR2	Phospholipid Scramblase 2	0.018	2.91
CD177	CD177 Molecule	0.005	2.79
BCL2L14	BCL2 Like 14	0.012	2.77
MRGPRE	MAS Related GPR Family Member E	0.007	2.58
CCL2	C-C Motif Chemokine Ligand 2	0.003	2.55
THSD7A	Thrombospondin Type 1 Domain Containing 7A	0.009	2.55
SIGLEC1	Sialic Acid Binding Ig Like Lectin 1	0.001	2.46
FBLIM1	Filamin Binding LIM Protein 1	0.035	2.43
SLC28A2	Solute Carrier Family 28 Member 2	0.038	2.42
DCN	Decorin	0.030	2.39
HESX1	HESX Homeobox 1	0.021	2.38
SLC7A4	Solute Carrier Family 7 Member 4	0.015	2.34
IFI44L	Interferon Induced Protein 44 Like	0.005	2.30
TPSAB1	Tryptase Alpha/Beta 1	0.012	2.29
IFI27	Interferon Alpha Inducible Protein 27	0.016	2.28
CCNA1	Cyclin A1	< 0.001	2.26
EPHA10	EPH Receptor A10	0.030	2.25
PRRG1	Proline Rich And Gla Domain 1	0.012	2.25

PROX1	Prospero Homeobox 1	0.049	2.25
RSAD2	Radical S-Adenosyl Methionine Domain Containing 2	0.018	2.20
CD163L1	CD163 Molecule Like 1	0.047	2.18
SFTPB	Surfactant Protein B	0.046	2.16
USP18	Ubiquitin Specific Peptidase 18	0.002	2.14
ISG15	ISG15 Ubiquitin Like Modifier	0.006	2.13
CFAP141	Cilia And Flagella Associated Protein 141	0.028	2.12
PARD3B	Par-3 Family Cell Polarity Regulator Beta	0.036	2.11
APOBEC3B	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3B	0.012	2.10
SPAG17	Sperm Associated Antigen 17	0.034	2.09
H2BE1	H2B.E Variant Histone 1	0.001	2.04
IFIT1	Interferon Induced Protein With Tetratricopeptide Repeats 1	0.012	2.04
DLC1	DLC1 Rho Gtpase Activating Protein	0.010	2.00
IFI44	Interferon Induced Protein 44	0.010	1.99
SPESP1	Sperm Equatorial Segment Protein 1	0.027	1.98
CXCL10	C-X-C Motif Chemokine Ligand 10	0.026	1.97
TMC4	Transmembrane Channel Like 4	< 0.001	1.96
OAS3	2'-5'-Oligoadenylate Synthetase 3	0.009	1.96
CMPK2	Cytidine/Uridine Monophosphate Kinase 2	0.013	1.95
VEPH1	Ventricular Zone Expressed PH Domain Containing 1	0.005	1.94
HPN	Hepsin	0.016	1.91
DZIP1L	DAZ Interacting Zinc Finger Protein 1 Like	0.012	1.90
PTGES3L	Prostaglandin E Synthase 3 Like	0.030	1.90
TMEM92	Transmembrane Protein 92	0.009	1.88
MX1	MX Dynamin Like Gtpase 1	0.004	1.87
BOK	BCL2 Family Apoptosis Regulator BOK	0.003	1.85
MMP8	Matrix Metallopeptidase 8	0.003	1.85
TEX101	Testis Expressed 101	0.032	1.85
PTH2R	Parathyroid Hormone 2 Receptor	0.012	1.84
PLSCR4	Phospholipid Scramblase 4	0.048	1.84

TMEM132C	Transmembrane Protein 132C	0.044	1.83
SLC8A2	Solute Carrier Family 8 Member A2	0.022	1.80
LAMP3	Lysosomal Associated Membrane Protein 3	0.025	1.79
SPATA1	Spermatogenesis Associated 1	0.005	1.79
IFI6	Interferon Alpha Inducible Protein 6	0.012	1.78
TRIM6	Tripartite Motif Containing 6	0.009	1.78
HERC5	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase 5	0.013	1.77
TLN2	Talin 2	0.038	1.77
CATSPERE	Catsper Channel Auxiliary Subunit Epsilon	0.010	1.76
BRCA2	BRCA2 DNA Repair Associated	0.014	1.75
MYH11	Myosin Heavy Chain 11	0.031	1.75
SERPINE1	Serpin Family E Member 1	0.002	1.73
C1QB	Complement C1q B Chain	0.048	1.72
MSR1	Macrophage Scavenger Receptor 1	0.010	1.71
CCDC194	Coiled-Coil Domain Containing 194	0.023	1.71
MCM10	Minichromosome Maintenance 10 Replication Initiation Factor	0.025	1.71
KIF7	Kinesin Family Member 7	0.007	1.71
AGRN	Agrin	0.003	1.70
EXOC3L1	Exocyst Complex Component 3 Like 1	0.019	1.69
IL31RA	Interleukin 31 Receptor A	0.003	1.69
KIF26A	Kinesin Family Member 26A	0.022	1.68
IFIT3	Interferon Induced Protein With Tetratricopeptide Repeats 3	0.029	1.68
CA6	Carbonic Anhydrase 6	0.045	1.68
ANO5	Anoctamin 5	0.005	1.68
SAMD4A	Sterile Alpha Motif Domain Containing 4A	0.002	1.67
TNFAIP6	TNF Alpha Induced Protein 6	0.011	1.67
NEXN	Nexilin F-Actin Binding Protein	0.020	1.66
ANGPT1	Angiopoietin 1	0.003	1.66
LRRC43	Leucine Rich Repeat Containing 43	0.030	1.66

PNMA5	PNMA Family Member 5	0.028	1.65
SELENOP	Selenoprotein P	0.041	1.65
IFIT2	Interferon Induced Protein With Tetratricopeptide Repeats 2	0.011	1.64
LRRC7	Leucine Rich Repeat Containing 7	0.046	1.64
LOC112267968	Uncharacterized LOC112267968	0.039	1.63
SPX	Spexin Hormone	0.005	1.63
OASL	2'-5'-Oligoadenylate Synthetase Like	0.027	1.62
RUFY4	RUN And FYVE Domain Containing 4	0.006	1.62
PIP5KL1	Phosphatidylinositol-4-Phosphate 5-Kinase Like 1	0.024	1.62
OAS2	2'-5'-Oligoadenylate Synthetase 2	0.013	1.62
LRRC37A	Leucine Rich Repeat Containing 37A	0.041	1.62
IL27	Interleukin 27	0.019	1.61
XCL1	X-C Motif Chemokine Ligand 1	0.003	1.61
OAS1	2'-5'-Oligoadenylate Synthetase 1	0.022	1.61
MIA	MIA SH3 Domain Containing	0.046	1.60
TTC26	Tetratricopeptide Repeat Domain 26	0.007	1.60
MARCO	Macrophage Receptor With Collagenous Structure	0.004	1.60
CLDN23	Claudin 23	0.001	1.58
RSPH9	Radial Spoke Head Component 9	0.027	1.58
IFITM3	Interferon Induced Transmembrane Protein 3	0.042	1.57
TRNP1	TMF1 Regulated Nuclear Protein 1	0.014	1.57
CXCL8	C-X-C Motif Chemokine Ligand 8	0.032	1.56
PLA2G7	Phospholipase A2 Group VII	< 0.001	1.56
ZNF684	Zinc Finger Protein 684	0.008	1.56
PLSCR1	Phospholipid Scramblase 1	0.004	1.55
PGAM2	Phosphoglycerate Mutase 2	0.003	1.54
GNAI1	G Protein Subunit Alpha I1	0.014	1.54
KIR3DL1	Killer Cell Immunoglobulin Like Receptor, Three Ig Domains And Long Cytoplasmic Tail 1	0.010	1.53

LGALS9B	Galectin 9B	0.012	1.53
HERC6	HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 6	0.003	1.53
PRR5-ARHGAP8	PRR5-ARHGAP8 Readthrough	0.045	1.53
TMEM51	Transmembrane Protein 51	0.018	1.53
CDKL1	Cyclin Dependent Kinase Like 1	0.004	1.53
SOX7	SRY-Box Transcription Factor 7	0.028	1.52
FAT4	FAT Atypical Cadherin 4	0.046	1.52
XYLB	Xylulokinase	0.020	1.52
DIXDC1	DIX Domain Containing 1	0.009	1.51
WNT5B	Wnt Family Member 5B	0.006	1.50
OBSL1	Obscurin Like Cytoskeletal Adaptor 1	0.030	1.50
GAS2L3	Growth Arrest Specific 2 Like 3	0.037	1.49
SPATC1	Spermatogenesis And Centriole Associated 1	0.043	1.49
TFEC	Transcription Factor EC	0.005	1.49
TLR3	Toll Like Receptor 3	0.014	1.48
EIF2AK2	Eukaryotic Translation Initiation Factor 2 Alpha Kinase 2	0.009	1.48
LRP6	LDL Receptor Related Protein 6	0.031	1.47
MT2A	Metallothionein 2A	0.043	1.47
SUCNR1	Succinate Receptor 1	0.041	1.46
IFIT5	Interferon Induced Protein With Tetratricopeptide Repeats 5	0.014	1.46
DDX60	Dexd/H-Box Helicase 60	0.020	1.46
CASP5	Caspase 5	0.033	1.45
C1R	Complement C1r	0.001	1.44
TENM1	Teneurin Transmembrane Protein 1	0.029	1.44
ZNF117	Zinc Finger Protein 117	0.001	1.44
LCN2	Lipocalin 2	0.019	1.44
HOXA9	Homeobox A9	0.028	1.43
ANKRD9	Ankyrin Repeat Domain 9	0.012	1.43

TDRP	Testis Development Related Protein	0.038	1.42
IL3RA	Interleukin 3 Receptor Subunit Alpha	0.004	1.42
BCAT1	Branched Chain Amino Acid Transaminase 1	0.046	1.42
TDRD9	Tudor Domain Containing 9	0.025	1.41
DDX60L	Dexd/H-Box 60 Like	0.007	1.41
FRMD3	FERM Domain Containing 3	0.031	1.41
TCN2	Transcobalamin 2	0.015	1.41
IFIH1	Interferon Induced With Helicase C Domain 1	0.009	1.41
HP	Haptoglobin	0.044	1.40
LRRC75B	Leucine Rich Repeat Containing 75B	0.017	1.40
CA8	Carbonic Anhydrase 8	0.039	1.40
ODF3B	Outer Dense Fiber Of Sperm Tails 3B	0.018	1.39
SNORC	Secondary Ossification Center Associated Regulator Of Chondrocyte Maturation	0.030	1.39
LAP3	Leucine Aminopeptidase 3	0.032	1.39
TNFSF10	TNF Superfamily Member 10	0.014	1.38
DHX58	Dexh-Box Helicase 58	0.018	1.38
SRGAP2B	SLIT-ROBO Rho Gtpase Activating Protein 2B	0.009	1.38
PGAP1	Post-GPI Attachment To Proteins Inositol Deacylase 1	0.037	1.37
ALDH8A1	Aldehyde Dehydrogenase 8 Family Member A1	0.019	1.37
CNTLN	Centlein	0.009	1.36
CDH26	Cadherin 26	0.039	1.36
IRF7	Interferon Regulatory Factor 7	0.029	1.36
GCSAML	Germinal Center Associated Signaling And Motility Like	0.048	1.36
BATF3	Basic Leucine Zipper ATF-Like Transcription Factor 3	0.020	1.36
NOMO3	NODAL Modulator 3	< 0.001	1.36
DDX58	Dexd/H-Box Helicase 58	0.037	1.36
TRPM4	Transient Receptor Potential Cation Channel Subfamily M Member 4	0.030	1.35
POMC	Proopiomelanocortin	0.017	1.35

DERPC	DERPC Proline And Glycine Rich Nuclear Protein	0.019	1.35
LRRC37A2	Leucine Rich Repeat Containing 37 Member A2	0.045	1.35
FLACC1	Flagellum Associated Containing Coiled-Coil Domains 1	0.037	1.34
LYVE1	Lymphatic Vessel Endothelial Hyaluronan Receptor 1	0.047	1.34
MX2	MX Dynamin Like Gtpase 2	0.011	1.34
SLC16A4	Solute Carrier Family 16 Member 4	0.043	1.34
H4C8	H4 Clustered Histone 8	0.033	1.34
SAMD9L	Sterile Alpha Motif Domain Containing 9 Like	0.033	1.34
FGD6	FYVE, Rhogef And PH Domain Containing 6	0.011	1.34
KIAA1958	Kiaa1958	0.035	1.34
TRIM22	Tripartite Motif Containing 22	0.039	1.33
PARP12	Poly(ADP-Ribose) Polymerase Family Member 12	0.015	1.33
SAMD9	Sterile Alpha Motif Domain Containing 9	0.023	1.33
SHB	SH2 Domain Containing Adaptor Protein B	0.017	1.33
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3A	0.011	1.32
FZD5	Frizzled Class Receptor 5	0.018	1.32
ZNF107	Zinc Finger Protein 107	0.004	1.32
ALDH1A1	Aldehyde Dehydrogenase 1 Family Member A1	0.006	1.32
ICA1L	Islet Cell Autoantigen 1 Like	0.041	1.31
FCGR2B	Fc Fragment Of Igg Receptor Iib	0.017	1.31
SDSL	Serine Dehydratase Like	0.043	1.31
AGBL2	AGBL Carboxypeptidase 2	0.036	1.31
CCDC24	Coiled-Coil Domain Containing 24	0.003	1.31
STEAP3	STEAP3 Metalloreductase	0.021	1.30
IL17RC	Interleukin 17 Receptor C	0.018	1.30
OSM	Oncostatin M	0.014	1.30
TNFSF13B	TNF Superfamily Member 13b	0.050	1.30
HPSE	Heparanase	0.010	1.30
CCDC62	Coiled-Coil Domain Containing 62	0.046	1.29
TREX1	Three Prime Repair Exonuclease 1	0.018	1.29

FOLR2	Folate Receptor Beta	0.014	1.29
PARP9	Poly(ADP-Ribose) Polymerase Family Member 9	0.039	1.29
SLFN12	Schlafen Family Member 12	0.028	1.29
IL1RN	Interleukin 1 Receptor Antagonist	0.035	1.29
KIF16B	Kinesin Family Member 16B	0.006	1.28
TMEM123	Transmembrane Protein 123	0.020	1.28
FLVCR2	FLVCR Heme Transporter 2	0.007	1.28
STON2	Stonin 2	0.044	1.28
ERMN	Ermin	0.021	1.28
SLC24A4	Solute Carrier Family 24 Member 4	0.017	1.27
RNF165	Ring Finger Protein 165	0.038	1.27
ATP8B4	Atpase Phospholipid Transporting 8B4 (Putative)	0.003	1.27
NT5C3A	5'-Nucleotidase, Cytosolic IIIA	0.029	1.27
TYMP	Thymidine Phosphorylase	0.036	1.27
MAFB	MAF Bzip Transcription Factor B	0.035	1.27
KIAA0895L	KIAA0895 Like	0.025	1.27
IL15RA	Interleukin 15 Receptor Subunit Alpha	0.008	1.27
SCARF1	Scavenger Receptor Class F Member 1	0.036	1.27
PLA2G4A	Phospholipase A2 Group IVA	0.017	1.26
CCL3	C-C Motif Chemokine Ligand 3	0.021	1.26
CCDC78	Coiled-Coil Domain Containing 78	0.025	1.26
KIAA1522	Kiaa1522	0.011	1.26
IFI16	Interferon Gamma Inducible Protein 16	0.009	1.26
ERV3-1	Endogenous Retrovirus Group 3 Member 1, Envelope	0.019	1.25
BST2	Bone Marrow Stromal Cell Antigen 2	0.037	1.25
STAT2	Signal Transducer And Activator Of Transcription 2	0.050	1.25
C9orf72	C9orf72-SMCR8 Complex Subunit	0.012	1.25
TRIM5	Tripartite Motif Containing 5	0.035	1.25
ZNF253	Zinc Finger Protein 253	0.027	1.25
RIN2	Ras And Rab Interactor 2	0.031	1.24

CD300E	CD300e Molecule	0.047	1.24
NPIPB11	Nuclear Pore Complex Interacting Protein Family Member B11	0.011	1.24
TMEM150B	Transmembrane Protein 150B	0.017	1.24
NETO2	Neuropilin And Tolloid Like 2	0.038	1.23
NEK3	NIMA Related Kinase 3	0.001	1.23
CPNE8	Copine 8	0.031	1.23
S1PR3	Sphingosine-1-Phosphate Receptor 3	0.023	1.23
PIK3C2A	Phosphatidylinositol-4-Phosphate 3-Kinase Catalytic Subunit Type 2 Alpha	0.044	1.23
H2BC21	H2B Clustered Histone 21	0.037	1.23
GPD2	Glycerol-3-Phosphate Dehydrogenase 2	0.034	1.23
LMO2	LIM Domain Only 2	0.013	1.22
RBM43	RNA Binding Motif Protein 43	0.011	1.22
CCDC149	Coiled-Coil Domain Containing 149	0.008	1.22
STX2	Syntaxin 2	0.025	1.22
MNDA	Myeloid Cell Nuclear Differentiation Antigen	0.012	1.22
PBLD	Phenazine Biosynthesis Like Protein Domain Containing	0.012	1.22
KCNMB1	Potassium Calcium-Activated Channel Subfamily M Regulatory Beta Subunit 1	0.036	1.22
JAK2	Janus Kinase 2	0.032	1.21
CREG1	Cellular Repressor Of E1A Stimulated Genes 1	0.002	1.21
KANK3	KN Motif And Ankyrin Repeat Domains 3	0.045	1.21
CCRL2	C-C Motif Chemokine Receptor Like 2	0.023	1.21
ZNF528	Zinc Finger Protein 528	0.045	1.21
MARCHF1	Membrane Associated Ring-CH-Type Finger 1	0.024	1.21
PHF11	PHD Finger Protein 11	0.007	1.21
UNC93B1	Unc-93 Homolog B1, TLR Signaling Regulator	0.025	1.21
ASGR1	Asialoglycoprotein Receptor 1	0.050	1.21
STAC3	SH3 And Cysteine Rich Domain 3	0.036	1.21
TSPAN4	Tetraspanin 4	0.003	1.20

CPVL	Carboxypeptidase Vitellogenic Like	0.014	1.20
FAM8A1	Family With Sequence Similarity 8 Member A1	0.020	1.20
CALML4	Calmodulin Like 4	0.019	1.20
SLC2A9	Solute Carrier Family 2 Member 9	0.003	1.20
P2RX7	Purinergic Receptor P2X 7	0.024	1.20
H2AC6	H2A Clustered Histone 6	0.026	1.20
HIBCH	3-Hydroxyisobutyryl-Coa Hydrolase	0.021	-1.20
RAB30	RAB30, Member RAS Oncogene Family	0.047	-1.20
CD79A	CD79a Molecule	0.039	-1.21
SEMA4F	Ssemaphorin 4F	0.004	-1.21
C16orf74	Chromosome 16 Open Reading Frame 74	0.024	-1.21
CD72	CD72 Molecule	0.045	-1.22
NME4	NME/NM23 Nucleoside Diphosphate Kinase 4	0.025	-1.22
PLXNA1	Plexin A1	0.017	-1.22
FBLN7	Fibulin 7	0.029	-1.22
NET1	Neuroepithelial Cell Transforming 1	0.039	-1.22
STRBP	Spermatid Perinuclear RNA Binding Protein	0.010	-1.22
CD19	CD19 Molecule	0.026	-1.23
PALD1	Phosphatase Domain Containing Paladin 1	0.032	-1.23
PMEPA1	Prostate Transmembrane Protein, Androgen Induced 1	0.038	-1.23
FAM241B	Family With Sequence Similarity 241 Member B	0.015	-1.23
NT5DC2	5'-Nucleotidase Domain Containing 2	0.049	-1.23
KLHL14	Kelch Like Family Member 14	0.034	-1.23
WFS1	Wolframin ER Transmembrane Glycoprotein	0.026	-1.23
LRFN3	Leucine Rich Repeat And Fibronectin Type III Domain Containing 3	0.029	-1.24
PRICKLE1	Prickle Planar Cell Polarity Protein 1	0.030	-1.24
LOC112694756	Uncharaterized LOC112694756	0.039	-1.24
PHACTR1	Phosphatase And Actin Regulator 1	0.036	-1.25

ZNF28	Zinc Finger Protein 28	0.040	-1.25
NBPF20	NBPF Member 20	0.034	-1.25
ENC1	Ectodermal-Neural Cortex 1	0.044	-1.25
RDH10	Retinol Dehydrogenase 10	0.006	-1.25
SLC9A7	Solute Carrier Family 9 Member A7	0.003	-1.26
KRTCAP3	Keratinocyte Associated Protein 3	0.022	-1.26
PDE7B	Phosphodiesterase 7B	0.032	-1.27
PPP1R13L	Protein Phosphatase 1 Regulatory Subunit 13 Like	0.025	-1.27
RTN4RL1	Reticulon 4 Receptor Like 1	0.047	-1.28
DPF3	Double PHD Fingers 3	0.043	-1.29
CBR3	Carbonyl Reductase 3	0.038	-1.30
SVIP	Small VCP Interacting Protein	0.043	-1.30
SPAG8	Sperm Associated Antigen 8	0.029	-1.30
WNT10A	Wnt Family Member 10A	0.010	-1.30
COBLL1	Cordon-Bleu WH2 Repeat Protein Like 1	0.024	-1.30
DTX1	Deltex E3 Ubiquitin Ligase 1	< 0.001	-1.31
ESR1	Estrogen Receptor 1	0.028	-1.31
SLC16A11	Solute Carrier Family 16 Member 11	0.040	-1.32
XPNPEP2	X-Prolyl Aminopeptidase 2	0.018	-1.32
AFAP1	Actin Filament Associated Protein 1	0.035	-1.33
EML5	EMAP Like 5	0.016	-1.33
ANKRD26	Ankyrin Repeat Domain 26	0.022	-1.34
RHOBTB3	Rho Related BTB Domain Containing 3	0.036	-1.34
FBXO2	F-Box Protein 2	0.006	-1.34
PKMYT1	Protein Kinase, Membrane Associated Tyrosine/Threonine 1	0.043	-1.35
CEP170B	Centrosomal Protein 170B	0.024	-1.35
SLC35F3	Solute Carrier Family 35 Member F3	0.025	-1.39
CCR9	C-C Motif Chemokine Receptor 9	0.044	-1.40
TCL1A	TCL1 Family AKT Coactivator A	0.013	-1.40
SLC16A14	Solute Carrier Family 16 Member 14	0.046	-1.41

MSC	Musculin	0.019	-1.42
GRIK5	Glutamate Ionotropic Receptor Kainate Type Subunit 5	0.024	-1.42
ZNF683	Zinc Finger Protein 683	0.007	-1.42
ACOT11	Acyl-Coa Thioesterase 11	0.042	-1.42
CCDC170	Coiled-Coil Domain Containing 170	0.006	-1.42
SEC14L2	SEC14 Like Lipid Binding 2	0.016	-1.42
MMP24	Matrix Metallopeptidase 24	0.029	-1.43
NRCAM	Neuronal Cell Adhesion Molecule	0.028	-1.43
CYP46A1	Cytochrome P450 Family 46 Subfamily A Member 1	0.012	-1.46
GLB1L2	Galactosidase Beta 1 Like 2	0.025	-1.47
EPHB3	EPH Receptor B3	0.032	-1.47
GPHN	Gephyrin	< 0.001	-1.47
GGT5	Gamma-Glutamyltransferase 5	0.036	-1.48
LCN12	Lipocalin 12	0.025	-1.48
KLC3	Kinesin Light Chain 3	0.041	-1.49
TTC39A	Tetratricopeptide Repeat Domain 39A	0.013	-1.49
ISM1	Isthmin 1	0.017	-1.50
PLLP	Plasmolipin	0.001	-1.51
PTPRF	Protein Tyrosine Phosphatase Receptor Type F	0.050	-1.51
RPL9	Ribosomal Protein L9	0.037	-1.51
ESPNL	Espin Like	0.023	-1.52
PMFBP1	Polyamine Modulated Factor 1 Binding Protein 1	0.013	-1.53
GSTA4	Glutathione S-Transferase Alpha 4	0.008	-1.54
APOLD1	Apolipoprotein L Domain Containing 1	0.019	-1.55
DHDH	Dihydrodiol Dehydrogenase	0.024	-1.56
REELD1	Reeler Domain Containing 1	0.010	-1.57
SLC5A10	Solute Carrier Family 5 Member 10	0.004	-1.57
ATP2A1	Atpase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 1	0.013	-1.57

KHDRBS3	KH RNA Binding Domain Containing, Signal Transduction Associated 3	0.034	-1.60
SNX32	Sorting Nexin 32	0.004	-1.60
SEMA5A	Semaphorin 5A	0.028	-1.61
CABP7	Calcium Binding Protein 7	0.036	-1.63
IL24	Interleukin 24	0.044	-1.65
ERVH48-1	Endogenous Retrovirus Group 48 Member 1	0.029	-1.66
IGDCC4	Immunoglobulin Superfamily DCC Subclass Member 4	0.013	-1.67
FSIP1	Fibrous Sheath Interacting Protein 1	0.049	-1.67
THRB	Thyroid Hormone Receptor Beta	0.016	-1.68
FAM78B	Family With Sequence Similarity 78 Member B	0.021	-1.68
CFAP251	Cilia And Flagella Associated Protein 251	0.007	-1.71
CPNE6	Copine 6	0.022	-1.71
PLA2G4C	Phospholipase A2 Group IVC	0.011	-1.73
FMN1	Formin 1	0.032	-1.74
TNNT1	Troponin T1, Slow Skeletal Type	0.001	-1.76
PDZD3	PDZ Domain Containing 3	0.018	-1.76
MYBPC2	Myosin Binding Protein C2	0.038	-1.77
CDH4	Cadherin 4	0.038	-1.77
SIGLEC12	Sialic Acid Binding Ig Like Lectin 12	0.023	-1.77
APC2	APC Regulator Of WNT Signaling Pathway 2	0.043	-1.79
TM4SF19	Transmembrane 4 L Six Family Member 19	0.009	-1.80
MAST1	Microtubule Associated Serine/Threonine Kinase 1	0.011	-1.81
TNS2	Tensin 2	0.022	-1.84
GABRD	Gamma-Aminobutyric Acid Type A Receptor Subunit Delta	0.025	-1.89
COL15A1	Collagen Type XV Alpha 1 Chain	0.023	-1.89
GRK7	G Protein-Coupled Receptor Kinase 7	0.024	-1.90
SLC2A12	Solute Carrier Family 2 Member 12	0.046	-1.93
PLPP2	Phospholipid Phosphatase 2	0.024	-1.93
LCA5	Lebercilin LCA5	0.033	-1.93

SCN11A	Sodium Voltage-Gated Channel Alpha Subunit 11	0.012	-1.97
PPARGC1A	PPARG Coactivator 1 Alpha	0.037	-1.98
TACSTD2	Tumor Associated Calcium Signal Transducer 2	0.010	-2.01
EFCAB1	EF-Hand Calcium Binding Domain 1	0.038	-2.01
CPA5	Carboxypeptidase A5	0.028	-2.02
CDK15	Cyclin Dependent Kinase 15	0.034	-2.03
CERKL	Ceramide Kinase Like	0.018	-2.09
LONRF2	LON Peptidase N-Terminal Domain And Ring Finger 2	0.046	-2.10
RPRML	Reprimo Like	0.040	-2.12
TENM4	Teneurin Transmembrane Protein 4	0.025	-2.14
EYA1	EYA Transcriptional Coactivator And Phosphatase 1	0.041	-2.15
IGSF9	Immunoglobulin Superfamily Member 9	0.015	-2.20
RAG1	Recombination Activating 1	0.001	-2.25
GRIP1	Glutamate Receptor Interacting Protein 1	0.001	-2.34
SMIM10L2B	Small Integral Membrane Protein 10 Like 2B	0.033	-2.40
SAXO2	Stabilizer Of Axonemal Microtubules 2	0.013	-2.47
PCDH8	Protocadherin 8	0.004	-2.50
MTRNR2L8	MT-RNR2 Like 8	0.003	-2.69
VPREB1	V-Set Pre-B Cell Surrogate Light Chain 1	0.016	-2.97
C4BPA	Complement Component 4 Binding Protein Alpha	0.006	-2.97
PCDHA4	Protocadherin Alpha 4	0.040	-3.04
C17orf50	Chromosome 17 Open Reading Frame 50	0.020	-3.10
MUC12	Mucin 12, Cell Surface Associated	0.041	-3.21
DMRTC1	DMRT Like Family C1	0.002	-3.67
RNF150	Ring Finger Protein 150	0.006	-4.42
GRIP2	Glutamate Receptor Interacting Protein 2	0.002	-5.49
CSH2	Chorionic Somatomammotropin Hormone 2	< 0.001	$-40x10^{5}$

Table 8E. List of 471 genes differently modulated in females MDD vs females LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05)

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3	TBC1 Domain Family Member 3	0.002	39.05
PRSS50	Serine Protease 50	0.004	4.66
SCGB3A1	Secretoglobin Family 3A Member 1	< 0.001	4.52
TM4SF1	Transmembrane 4 L Six Family Member 1	0.017	3.95
RP1L1	RP1 Like 1	0.044	3.10
HBG2	Hemoglobin Subunit Gamma 2	0.013	2.72
DNAH12	Dynein Axonemal Heavy Chain 12	0.015	2.64
CA12	Carbonic Anhydrase 12	0.006	2.56
ADCY10	Adenylate Cyclase 10	0.010	2.52
INAVA	Innate Immunity Activator	0.004	2.51
KIF26A	Kinesin Family Member 26A	0.006	2.47
VSIG8	V-Set And Immunoglobulin Domain Containing 8	0.011	2.37
FAM72C	Family With Sequence Similarity 72 Member C	0.045	2.32
SFTPB	Surfactant Protein B	0.036	2.31
COL16A1	Collagen Type XVI Alpha 1 Chain	0.049	2.30
SPESP1	Sperm Equatorial Segment Protein 1	0.013	2.28
PLSCR4	Phospholipid Scramblase 4	0.018	2.22
DNAH7	Dynein Axonemal Heavy Chain 7	0.033	2.20
GPRC5C	G Protein-Coupled Receptor Class C Group 5 Member C	0.011	2.13
DRC3	Dynein Regulatory Complex Subunit 3	0.032	2.02
CIART	Circadian Associated Repressor Of Transcription	0.025	1.91
CATSPERE	Catsper Channel Auxiliary Subunit Epsilon	0.006	1.91
CBS	Cystathionine Beta-Synthase	0.003	1.90
SHANK2	SH3 And Multiple Ankyrin Repeat Domains 2	0.028	1.89
CERS3	Ceramide Synthase 3	0.036	1.88
HPN	Hepsin	0.022	1.84

CORO7-PAM16	CORO7-PAM16 Readthrough	0.022	1.80
H2BC17	H2B Clustered Histone 17	0.014	1.76
KIF7	Kinesin Family Member 7	0.020	1.76
DNAH17	Dynein Axonemal Heavy Chain 17	0.049	1.74
TMEM92	Transmembrane Protein 92	0.026	1.73
OXTR	Oxytocin Receptor	0.022	1.73
U2AF1	U2 Small Nuclear RNA Auxiliary Factor 1	0.024	1.73
DZIP1L	DAZ Interacting Zinc Finger Protein 1 Like	0.044	1.71
ITGA9	Integrin Subunit Alpha 9	0.014	1.69
RASL10A	RAS Like Family 10 Member A	0.010	1.68
SEMG1	Semenogelin 1	0.036	1.68
TMC4	Transmembrane Channel Like 4	0.003	1.66
KIF4A	Kinesin Family Member 4A	0.033	1.65
PRRG2	Proline Rich And Gla Domain 2	0.037	1.64
CYB5R2	Cytochrome B5 Reductase 2	0.027	1.64
CLEC18B	C-Type Lectin Domain Family 18 Member B	0.028	1.63
GRIN3B	Glutamate Ionotropic Receptor NMDA Type Subunit 3B	0.018	1.61
DUSP13	Dual Specificity Phosphatase 13	0.044	1.61
OSCP1	Organic Solute Carrier Partner 1	0.049	1.60
TNFAIP6	TNF Alpha Induced Protein 6	0.025	1.59
SFN	Stratifin	0.013	1.59
WWTR1	WW Domain Containing Transcription Regulator 1	0.031	1.58
CCNA1	Cyclin A1	0.049	1.54
ALPK3	Alpha Kinase 3	0.010	1.52
H2AC8	H2A Clustered Histone 8	0.015	1.51
C5orf34	Chromosome 5 Open Reading Frame 34	0.016	1.49
GBP6	Guanylate Binding Protein Family Member 6	0.029	1.48
MGAM2	Maltase-Glucoamylase 2 (Putative)	0.034	1.47
LYG1	Lysozyme G1	0.002	1.46

LOC400499	Putative Uncharacterized Protein LOC400499	0.001	1.46
EDA	Ectodysplasin A	0.044	1.46
DCAF4L1	DDB1 And CUL4 Associated Factor 4 Like 1	0.007	1.46
RSPH9	Radial Spoke Head Component 9	0.048	1.44
CFAP45	Cilia And Flagella Associated Protein 45	0.008	1.44
SUV39H2	SUV39H2 Histone Lysine Methyltransferase	0.023	1.44
C4orf50	Chromosome 4 Open Reading Frame 50	0.045	1.44
COL18A1	Collagen Type XVIII Alpha 1 Chain	0.005	1.43
C1R	Complement C1r	0.002	1.43
LSMEM1	Leucine Rich Single-Pass Membrane Protein 1	0.013	1.42
FAM174B	Family With Sequence Similarity 174 Member B	0.008	1.42
GCM1	Glial Cells Missing Transcription Factor 1	0.007	1.41
FANCM	FA Complementation Group M	0.036	1.41
SCARF1	Scavenger Receptor Class F Member 1	0.003	1.40
GABRR2	Gamma-Aminobutyric Acid Type A Receptor Subunit Rho2	0.014	1.40
INSL3	Insulin Like 3	0.010	1.39
H2BC8	H2B Clustered Histone 8	0.019	1.39
PTGFRN	Prostaglandin F2 Receptor Inhibitor	0.043	1.38
PIGB	Phosphatidylinositol Glycan Anchor Biosynthesis Class B	0.001	1.37
VNN1	Vanin 1	0.040	1.37
H2BC21	H2B Clustered Histone 21	< 0.001	1.36
S1PR3	Sphingosine-1-Phosphate Receptor 3	0.001	1.36
PRRG4	Proline Rich And Gla Domain 4	0.012	1.36
ASPRV1	Aspartic Peptidase Retroviral Like 1	0.022	1.36
HOXA1	Homeobox A1	0.027	1.35
CRABP2	Cellular Retinoic Acid Binding Protein 2	0.047	1.35
GGT1	Gamma-Glutamyltransferase 1	0.011	1.34
PLAU	Plasminogen Activator, Urokinase	0.035	1.34
HCAR3	Hydroxycarboxylic Acid Receptor 3	0.004	1.34

NHLH1	Nescient Helix-Loop-Helix 1	0.018	1.34
IL1RN	Interleukin 1 Receptor Antagonist	0.009	1.34
BORCS8-MEF2B	BORCS8-MEF2B Readthrough	0.049	1.33
IL3RA	Interleukin 3 Receptor Subunit Alpha	0.024	1.33
FZD5	Frizzled Class Receptor 5	0.030	1.33
EEF1AKMT3	EEF1A Lysine Methyltransferase 3	0.016	1.33
ADM	Adrenomedullin	0.036	1.32
GHRL	Ghrelin And Obestatin Prepropeptide	0.008	1.32
HSPA1B	Heat Shock Protein Family A (Hsp70) Member 1B	0.003	1.32
NFIL3	Nuclear Factor, Interleukin 3 Regulated	0.002	1.32
UBE2D1	Ubiquitin Conjugating Enzyme E2 D1	0.021	1.32
TLR2	Toll Like Receptor 2	0.003	1.32
SMAD1	SMAD Family Member 1	0.031	1.32
TAFA2	TAFA Chemokine Like Family Member 2	0.047	1.31
PLGLB1	Plasminogen Like B1	0.037	1.31
ZCCHC2	Zinc Finger CCHC-Type Containing 2	0.045	1.31
CLEC1A	C-Type Lectin Domain Family 1 Member A	0.036	1.31
LOXL2	Lysyl Oxidase Like 2	0.022	1.31
ACSL1	Acyl-Coa Synthetase Long Chain Family Member 1	0.026	1.31
HCAR2	Hydroxycarboxylic Acid Receptor 2	0.006	1.31
B4GALT5	Beta-1,4-Galactosyltransferase 5	0.004	1.30
TRPM6	Transient Receptor Potential Cation Channel Subfamily M Member 6	0.029	1.30
ATP8B4	Atpase Phospholipid Transporting 8B4 (Putative)	0.018	1.30
FLVCR1	FLVCR Heme Transporter 1	0.010	1.30
RRP12	Ribosomal RNA Processing 12 Homolog	0.014	1.30
ZNF107	Zinc Finger Protein 107	0.018	1.29
SIPA1L2	Signal Induced Proliferation Associated 1 Like 2	0.011	1.29
CPT1B	Carnitine Palmitoyltransferase 1B	0.023	1.29

WDFY3	WD Repeat And FYVE Domain Containing 3	0.019	1.29
MORN3	MORN Repeat Containing 3	0.032	1.29
C10orf105	Chromosome 10 Open Reading Frame 105	0.002	1.29
H2BC4	H2B Clustered Histone 4	0.005	1.29
KIAA0319	Kiaa0319	0.017	1.29
CEP126	Centrosomal Protein 126	0.037	1.28
GPR27	G Protein-Coupled Receptor 27	0.016	1.28
HSPA6	Heat Shock Protein Family A (Hsp70) Member 6	0.007	1.28
LRG1	Leucine Rich Alpha-2-Glycoprotein 1	0.022	1.28
MX2	MX Dynamin Like Gtpase 2	0.031	1.28
CEP19	Centrosomal Protein 19	0.013	1.28
MARCKS	Myristoylated Alanine Rich Protein Kinase C Substrate	0.013	1.28
SIGLEC5	Sialic Acid Binding Ig Like Lectin 5	0.018	1.28
TRIP6	Thyroid Hormone Receptor Interactor 6	< 0.001	1.27
SLC22A4	Solute Carrier Family 22 Member 4	0.026	1.27
AVIL	Advillin	0.017	1.27
FFAR2	Free Fatty Acid Receptor 2	0.022	1.27
REM2	RRAD And GEM Like Gtpase 2	0.004	1.27
SH3GLB1	SH3 Domain Containing GRB2 Like, Endophilin B1	0.008	1.27
GLS2	Glutaminase 2	0.028	1.27
TMEM140	Transmembrane Protein 140	0.008	1.27
SERF1A	Small EDRK-Rich Factor 1A	0.022	1.26
KCNJ15	Potassium Inwardly Rectifying Channel Subfamily J Member 15	0.042	1.26
PMM2	Phosphomannomutase 2	0.043	1.26
KATNBL1	Katanin Regulatory Subunit B1 Like 1	0.048	1.26
HPSE	Heparanase	0.022	1.26
BMP6	Bone Morphogenetic Protein 6	0.043	1.26
RNASET2	Ribonuclease T2	0.005	1.26
DNA2	DNA Replication Helicase/Nuclease 2	0.044	1.25
DRAM1	DNA Damage Regulated Autophagy Modulator 1	0.024	1.25

CTBS	Chitobiase	0.015	1.25
FAM8A1	Family With Sequence Similarity 8 Member A1	0.025	1.25
FCGR2A	Fc Fragment Of Igg Receptor Iia	0.004	1.25
MANSC1	MANSC Domain Containing 1	0.050	1.25
CITED4	Cbp/P300 Interacting Transactivator With Glu/Asp Rich Carboxy-Terminal Domain 4	0.017	1.25
FTH1	Ferritin Heavy Chain 1	0.003	1.25
MCUR1	Mitochondrial Calcium Uniporter Regulator 1	0.026	1.25
TTLL4	Tubulin Tyrosine Ligase Like 4	0.010	1.24
UHRF1BP1L	UHRF1 Binding Protein 1 Like	0.020	1.24
ARL11	ADP Ribosylation Factor Like Gtpase 11	0.031	1.24
INAFM1	Inaf Motif Containing 1	0.004	1.24
CEP63	Centrosomal Protein 63	0.011	1.24
MOSPD2	Motile Sperm Domain Containing 2	0.030	1.24
ALPK1	Alpha Kinase 1	0.020	1.24
IMPA2	Inositol Monophosphatase 2	0.031	1.24
PSRC1	Proline And Serine Rich Coiled-Coil 1	0.030	1.24
PPP2R5A	Protein Phosphatase 2 Regulatory Subunit B'alpha	0.008	1.23
SERPINA1	Serpin Family A Member 1	0.021	1.23
CLDN9	Claudin 9	0.029	1.23
TMPRSS13	Transmembrane Serine Protease 13	0.046	1.23
CPPED1	Calcineurin Like Phosphoesterase Domain Containing 1	0.009	1.23
ARHGAP26	Rho Gtpase Activating Protein 26	0.003	1.23
SIRPA	Signal Regulatory Protein Alpha	0.006	1.23
IL1B	Interleukin 1 Beta	0.031	1.23
GK	Glycerol Kinase	0.025	1.23
FUT7	Fucosyltransferase 7	0.026	1.23
SECTM1	Secreted And Transmembrane 1	0.022	1.23
BACH1	BTB Domain And CNC Homolog 1	0.030	1.23

GNAQ	G Protein Subunit Alpha Q	0.026	1.23
CXCR1	C-X-C Motif Chemokine Receptor 1	0.014	1.22
CPNE2	Copine 2	0.006	1.22
CXCR2	C-X-C Motif Chemokine Receptor 2	0.012	1.22
GBGT1	Globoside Alpha-1,3-N-Acetylgalactosaminyltransferase 1 (FORS Blood Group)	0.004	1.22
APOBEC3A	Apolipoprotein B Mrna Editing Enzyme Catalytic Subunit 3A	0.037	1.22
CXCL16	C-X-C Motif Chemokine Ligand 16	0.035	1.22
EBPL	EBP Like	0.017	1.22
FBXL5	F-Box And Leucine Rich Repeat Protein 5	0.040	1.22
FRAT2	FRAT Regulator Of WNT Signaling Pathway 2	0.036	1.22
ST8SIA4	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 4	0.023	1.22
TREM1	Triggering Receptor Expressed On Myeloid Cells 1	0.025	1.22
RHOB	Ras Homolog Family Member B	0.004	1.22
SESN3	Sestrin 3	0.030	1.22
LIMK2	LIM Domain Kinase 2	0.033	1.22
FAM241A	Family With Sequence Similarity 241 Member A	0.016	1.22
IFI16	Interferon Gamma Inducible Protein 16	0.033	1.22
ITM2B	Integral Membrane Protein 2B	0.006	1.22
RICTOR	RPTOR Independent Companion Of MTOR Complex 2	0.048	1.21
PEAK3	PEAK Family Member 3	0.046	1.21
LPAR2	Lysophosphatidic Acid Receptor 2	0.022	1.21
MYD88	MYD88 Innate Immune Signal Transduction Adaptor	0.003	1.21
DCAF10	DDB1 And CUL4 Associated Factor 10	0.029	1.21
SLC19A1	Solute Carrier Family 19 Member 1	0.017	1.21
CPD	Carboxypeptidase D	0.037	1.21
SLC9A8	Solute Carrier Family 9 Member A8	0.002	1.21
FNIP1	Folliculin Interacting Protein 1	0.021	1.21
CSF2RB	Colony Stimulating Factor 2 Receptor Subunit Beta	0.011	1.21

APBB1IP	Amyloid Beta Precursor Protein Binding Family B Member 1 Interacting Protein	0.028	1.20
MCTP2	Multiple C2 And Transmembrane Domain Containing 2	0.044	1.20
PIM3	Pim-3 Proto-Oncogene, Serine/Threonine Kinase	0.006	1.20
B9D2	B9 Domain Containing 2	0.008	1.20
IQCN	IQ Motif Containing N	0.048	1.20
LILRA2	Leukocyte Immunoglobulin Like Receptor A2	0.021	1.20
AP5B1	Adaptor Related Protein Complex 5 Subunit Beta 1	0.015	1.20
PICALM	Phosphatidylinositol Binding Clathrin Assembly Protein	0.013	1.20
ACOX1	Acyl-Coa Oxidase 1	0.021	1.20
IGSF6	Immunoglobulin Superfamily Member 6	0.019	1.20
GNG5	G Protein Subunit Gamma 5	0.009	1.20
ADAL	Adenosine Deaminase Like	0.025	-1.20
RPE	Ribulose-5-Phosphate-3-Epimerase	0.031	-1.20
TFAP2E	Transcription Factor AP-2 Epsilon	0.039	-1.20
ZNF124	Zinc Finger Protein 124	0.018	-1.21
CENPH	Centromere Protein H	0.008	-1.22
DTX1	Deltex E3 Ubiquitin Ligase 1	0.034	-1.22
FCRLA	Fc Receptor Like A	0.035	-1.22
SLFN13	Schlafen Family Member 13	0.020	-1.22
ZCCHC18	Zinc Finger CCHC-Type Containing 18	0.032	-1.22
ACACB	Acetyl-Coa Carboxylase Beta	0.023	-1.22
SIPA1L3	Signal Induced Proliferation Associated 1 Like 3	0.007	-1.23
ZNF599	Zinc Finger Protein 599	0.015	-1.23
C1orf74	Chromosome 1 Open Reading Frame 74	0.013	-1.23
ABCB1	ATP Binding Cassette Subfamily B Member 1	0.026	-1.23
ZNF670	Zinc Finger Protein 670	0.047	-1.23
NPTXR	Neuronal Pentraxin Receptor	0.013	-1.23
ARHGAP32	Rho Gtpase Activating Protein 32	0.010	-1.23
ACSL6	Acyl-Coa Synthetase Long Chain Family Member 6	0.004	-1.24

ZNF567	Zinc Finger Protein 567	0.035	-1.24
SV2A	Synaptic Vesicle Glycoprotein 2A	0.044	-1.24
SLC9A7	Solute Carrier Family 9 Member A7	0.031	-1.24
TAF1A	TATA-Box Binding Protein Associated Factor, RNA Polymerase I Subunit A	0.020	-1.24
MTSS1	MTSS I-BAR Domain Containing 1	0.002	-1.24
ENC1	Ectodermal-Neural Cortex 1	0.048	-1.25
TCEA3	Transcription Elongation Factor A3	0.008	-1.25
TMEM38B	Transmembrane Protein 38B	0.045	-1.25
ERICH1	Glutamate Rich 1	0.002	-1.25
OTUD3	OTU Deubiquitinase 3	0.035	-1.25
ARL16	ADP Ribosylation Factor Like Gtpase 16	0.024	-1.25
KLHL3	Kelch Like Family Member 3	0.016	-1.25
PCDH9	Protocadherin 9	0.040	-1.25
PPP1R13L	Protein Phosphatase 1 Regulatory Subunit 13 Like	0.043	-1.26
ZC3H8	Zinc Finger CCCH-Type Containing 8	0.009	-1.26
CBX8	Chromobox 8	0.018	-1.26
RBM41	RNA Binding Motif Protein 41	0.020	-1.26
KDM8	Lysine Demethylase 8	0.002	-1.26
FBXO4	F-Box Protein 4	0.028	-1.27
FEZ1	Fasciculation And Elongation Protein Zeta 1	0.022	-1.27
DHCR24	24-Dehydrocholesterol Reductase	0.001	-1.27
TMA16	Translation Machinery Associated 16 Homolog	0.003	-1.27
B3GNT7	UDP-Glcnac:Betagal Beta-1,3-N-Acetylglucosaminyltransferase 7	0.038	-1.28
LOC102724428	Salt Inducible Kinase 1B (Putative)	0.030	-1.28
ZNF565	Zinc Finger Protein 565	0.019	-1.28
ZNF570	Zinc Finger Protein 570	0.014	-1.29
ZNF69	Zinc Finger Protein 69	0.006	-1.29
CTSW	Cathepsin W	0.027	-1.29

ZNF320	Zinc Finger Protein 320	0.026	-1.29
STRBP	Spermatid Perinuclear RNA Binding Protein	0.002	-1.29
PDE7B	Phosphodiesterase 7B	0.032	-1.29
ERBB2	Erb-B2 Receptor Tyrosine Kinase 2	0.028	-1.29
PTCH1	Patched 1	0.015	-1.29
CDHR1	Cadherin Related Family Member 1	0.042	-1.30
ADD2	Adducin 2	0.012	-1.30
NFYB	Nuclear Transcription Factor Y Subunit Beta	0.041	-1.30
DLG5	Discs Large MAGUK Scaffold Protein 5	0.040	-1.30
DISP1	Dispatched RND Transporter Family Member 1	0.027	-1.30
SLC16A11	Solute Carrier Family 16 Member 11	0.044	-1.30
KCNQ5	Potassium Voltage-Gated Channel Subfamily Q Member 5	0.017	-1.30
B4GALT7	Beta-1,4-Galactosyltransferase 7	0.028	-1.31
PTPRK	Protein Tyrosine Phosphatase Receptor Type K	0.045	-1.31
TCL1A	TCL1 Family AKT Coactivator A	0.024	-1.31
PRICKLE1	Prickle Planar Cell Polarity Protein 1	0.015	-1.31
BCAR3	BCAR3 Adaptor Protein, NSP Family Member	0.027	-1.31
BBS7	Bardet-Biedl Syndrome 7	0.034	-1.31
MICAL3	Microtubule Associated Monooxygenase, Calponin And LIM Domain Containing 3	0.012	-1.31
SARDH	Sarcosine Dehydrogenase	0.039	-1.32
ITGAV	Integrin Subunit Alpha V	0.047	-1.32
ZIK1	Zinc Finger Protein Interacting With K Protein 1	0.001	-1.32
ARVCF	ARVCF Delta Catenin Family Member	0.015	-1.32
USP51	Ubiquitin Specific Peptidase 51	0.034	-1.33
MCF2L	MCF.2 Cell Line Derived Transforming Sequence Like	0.003	-1.33
NET1	Neuroepithelial Cell Transforming 1	0.003	-1.33
ST8SIA6	ST8 Alpha-N-Acetyl-Neuraminide Alpha-2,8-Sialyltransferase 6	0.018	-1.33
ESR1	Estrogen Receptor 1	0.041	-1.34
EML5	EMAP Like 5	0.032	-1.34

FAM131B	Family With Sequence Similarity 131 Member B	0.049	-1.34
TMEM126A	Transmembrane Protein 126A	0.050	-1.34
BEND5	BEN Domain Containing 5	0.029	-1.34
SERPINE2	Serpin Family E Member 2	0.025	-1.35
ANKRD26	Ankyrin Repeat Domain 26	0.008	-1.35
FAM222A	Family With Sequence Similarity 222 Member A	0.042	-1.35
MRAS	Muscle RAS Oncogene Homolog	0.019	-1.35
GPR153	G Protein-Coupled Receptor 153	0.046	-1.35
CNN3	Calponin 3	0.030	-1.35
EFHD1	EF-Hand Domain Family Member D1	0.050	-1.35
KCNA2	Potassium Voltage-Gated Channel Subfamily A Member 2	0.042	-1.35
RGS9	Regulator Of G Protein Signaling 9	0.009	-1.35
NR4A1	Nuclear Receptor Subfamily 4 Group A Member 1	0.023	-1.36
FAM153A	Family With Sequence Similarity 153 Member A	0.010	-1.36
PAK6	P21 (RAC1) Activated Kinase 6	0.044	-1.37
BDH2	3-Hydroxybutyrate Dehydrogenase 2	0.034	-1.37
LOC102724594	U2 Small Nuclear RNA Auxiliary Factor 1 Like 5	0.019	-1.38
SMIM24	Small Integral Membrane Protein 24	0.043	-1.38
ZNRF3	Zinc And Ring Finger 3	0.033	-1.38
BFSP1	Beaded Filament Structural Protein 1	0.029	-1.39
SHF	Src Homology 2 Domain Containing F	0.033	-1.39
CBR3	Carbonyl Reductase 3	0.006	-1.39
MACROD2	Mono-ADP Ribosylhydrolase 2	0.018	-1.40
ZNF365	Zinc Finger Protein 365	0.036	-1.40
BEX2	Brain Expressed X-Linked 2	0.024	-1.40
AIFM3	Apoptosis Inducing Factor Mitochondria Associated 3	0.005	-1.40
ZACN	Zinc Activated Ion Channel	0.042	-1.41
ZFP2	ZFP2 Zinc Finger Protein	0.044	-1.41

KLRC4	Killer Cell Lectin Like Receptor C4	0.043	-1.41
B3GAT1	Beta-1,3-Glucuronyltransferase 1	0.049	-1.41
ITGA7	Integrin Subunit Alpha 7	0.044	-1.41
KIR3DL2	Killer Cell Immunoglobulin Like Receptor, Three Ig Domains And Long Cytoplasmic Tail 2	0.030	-1.41
RAB30	RAB30, Member RAS Oncogene Family	0.003	-1.42
FGFBP2	Fibroblast Growth Factor Binding Protein 2	0.021	-1.42
LAMC1	Laminin Subunit Gamma 1	0.011	-1.42
NCAM1	Neural Cell Adhesion Molecule 1	0.003	-1.42
SNX32	Sorting Nexin 32	0.040	-1.42
MTCL1	Microtubule Crosslinking Factor 1	0.030	-1.43
MTMR8	Myotubularin Related Protein 8	0.023	-1.43
TXNRD3	Thioredoxin Reductase 3	0.010	-1.44
SYBU	Syntabulin	0.021	-1.45
PRR29	Proline Rich 29	0.031	-1.45
MAP2	Microtubule Associated Protein 2	0.038	-1.45
TUBB3	Tubulin Beta 3 Class III	0.044	-1.45
PALS2	Protein Associated With LIN7 2, MAGUK Family Member	0.015	-1.45
DPF3	Double PHD Fingers 3	0.008	-1.46
LRP5	LDL Receptor Related Protein 5	0.020	-1.46
TMSB15B	Thymosin Beta 15B	0.010	-1.46
HPCA	Hippocalcin	0.036	-1.46
CDKN1C	Cyclin Dependent Kinase Inhibitor 1C	0.022	-1.46
RAB38	RAB38, Member RAS Oncogene Family	0.015	-1.46
HOMER1	Homer Scaffold Protein 1	0.031	-1.46
ADGRA3	Adhesion G Protein-Coupled Receptor A3	0.003	-1.48
COBLL1	Cordon-Bleu WH2 Repeat Protein Like 1	0.007	-1.48
MMEL1	Membrane Metalloendopeptidase Like 1	0.049	-1.48
FAM110C	Family With Sequence Similarity 110 Member C	0.035	-1.49

ICAM4	Intercellular Adhesion Molecule 4 (Landsteiner-Wiener Blood Group)	0.004	-1.49
GOLGA8R	Golgin A8 Family Member R	0.020	-1.50
CCR9	C-C Motif Chemokine Receptor 9	0.004	-1.50
PMFBP1	Polyamine Modulated Factor 1 Binding Protein 1	0.022	-1.51
NTRK2	Neurotrophic Receptor Tyrosine Kinase 2	0.036	-1.52
EAF2	ELL Associated Factor 2	0.045	-1.52
TTN	Titin	0.027	-1.53
PRSS23	Serine Protease 23	0.004	-1.54
PPP2R2C	Protein Phosphatase 2 Regulatory Subunit Bgamma	0.024	-1.55
JAG2	Jagged Canonical Notch Ligand 2	0.015	-1.56
SOBP	Sine Oculis Binding Protein Homolog	0.018	-1.56
DPPA4	Developmental Pluripotency Associated 4	0.006	-1.56
UGGT2	UDP-Glucose Glycoprotein Glucosyltransferase 2	0.031	-1.57
ZNF883	Zinc Finger Protein 883	0.008	-1.57
RPL9	Ribosomal Protein L9	0.002	-1.58
SLC5A10	Solute Carrier Family 5 Member 10	0.004	-1.59
DEGS2	Delta 4-Desaturase, Sphingolipid 2	0.012	-1.59
KHDRBS3	KH RNA Binding Domain Containing, Signal Transduction Associated 3	0.022	-1.59
MYO1A	Myosin IA	0.040	-1.59
P3H2	Prolyl 3-Hydroxylase 2	0.023	-1.59
ZMYND12	Zinc Finger MYND-Type Containing 12	0.036	-1.59
MFSD6L	Major Facilitator Superfamily Domain Containing 6 Like	0.041	-1.59
ANKRD31	Ankyrin Repeat Domain 31	0.044	-1.59
MYO6	Myosin VI	0.003	-1.60
CYTL1	Cytokine Like 1	0.044	-1.61
FGF9	Fibroblast Growth Factor 9	0.049	-1.61
B4GALNT4	Beta-1,4-N-Acetyl-Galactosaminyltransferase 4	0.015	-1.61
CETN3	Centrin 3	0.020	-1.61

ADAMTS6	ADAM Metallopeptidase With Thrombospondin Type 1 Motif 6	0.020	-1.63
RET	Ret Proto-Oncogene	0.016	-1.63
MYH10	Myosin Heavy Chain 10	0.036	-1.63
TMC2	Transmembrane Channel Like 2	0.027	-1.64
NCS1	Neuronal Calcium Sensor 1	0.020	-1.64
PLS3	Plastin 3	0.021	-1.67
STAC2	SH3 And Cysteine Rich Domain 2	0.015	-1.67
AKR1E2	Aldo-Keto Reductase Family 1 Member E2	0.038	-1.68
SEMA4G	Semaphorin 4G	0.021	-1.68
SERF1A	Small EDRK-Rich Factor 1A	0.011	-1.69
ADGRL3	Adhesion G Protein-Coupled Receptor L3	0.040	-1.69
TEDC2	Tubulin Epsilon And Delta Complex 2	0.037	-1.69
BCAM	Basal Cell Adhesion Molecule (Lutheran Blood Group)	0.026	-1.69
TMEM132A	Transmembrane Protein 132A	0.042	-1.69
CDC25C	Cell Division Cycle 25C	0.044	-1.69
PNMA6A	PNMA Family Member 6A	0.043	-1.70
KLRC2	Killer Cell Lectin Like Receptor C2	0.040	-1.71
FGFR4	Fibroblast Growth Factor Receptor 4	0.004	-1.71
CTTNBP2	Cortactin Binding Protein 2	0.025	-1.72
TTLL10	Tubulin Tyrosine Ligase Like 10	0.044	-1.72
GLIPR1L2	GLIPR1 Like 2	0.042	-1.72
DCLK1	Doublecortin Like Kinase 1	0.015	-1.73
IGSF3	Immunoglobulin Superfamily Member 3	0.047	-1.74
RBFOX2	RNA Binding Fox-1 Homolog 2	0.004	-1.74
SRCIN1	SRC Kinase Signaling Inhibitor 1	0.013	-1.75
ADGRF3	Adhesion G Protein-Coupled Receptor F3	0.044	-1.75
ADCY2	Adenylate Cyclase 2	0.044	-1.75
PROM2	Prominin 2	0.039	-1.76
PDGFRB	Platelet Derived Growth Factor Receptor Beta	0.003	-1.76

INHBA	Inhibin Subunit Beta A	0.041	-1.76
TFCP2L1	Transcription Factor CP2 Like 1	0.002	-1.77
SFRP2	Secreted Frizzled Related Protein 2	0.012	-1.77
PTPRG	Protein Tyrosine Phosphatase Receptor Type G	0.008	-1.79
ANKRD63	Ankyrin Repeat Domain 63	0.023	-1.79
LRRC63	Leucine Rich Repeat Containing 63	0.038	-1.81
BNC2	Basonuclin 2	0.005	-1.82
ACKR2	Atypical Chemokine Receptor 2	0.042	-1.82
NRCAM	Neuronal Cell Adhesion Molecule	< 0.001	-1.82
RALYL	RALY RNA Binding Protein Like	0.033	-1.83
CCDC42	Coiled-Coil Domain Containing 42	0.031	-1.84
ADRA1B	Adrenoceptor Alpha 1B	0.050	-1.84
LIMCH1	LIM And Calponin Homology Domains 1	0.022	-1.85
KNDC1	Kinase Non-Catalytic C-Lobe Domain Containing 1	0.002	-1.87
RASIP1	Ras Interacting Protein 1	0.039	-1.89
CDK15	Cyclin Dependent Kinase 15	0.046	-1.90
TMEM200B	Transmembrane Protein 200B	0.016	-1.92
CDH4	Cadherin 4	0.011	-1.93
PTX4	Pentraxin 4	0.022	-1.93
AIF1L	Allograft Inflammatory Factor 1 Like	0.042	-1.94
ARHGEF28	Rho Guanine Nucleotide Exchange Factor 28	0.009	-1.94
LINGO2	Leucine Rich Repeat And Ig Domain Containing 2	0.005	-1.95
EVPL	Envoplakin	0.006	-1.96
GPR20	G Protein-Coupled Receptor 20	0.009	-1.96
TGFBR3L	Transforming Growth Factor Beta Receptor 3 Like	0.047	-1.97
FHAD1	Forkhead Associated Phosphopeptide Binding Domain 1	0.027	-1.97
UNC5B	Unc-5 Netrin Receptor B	0.014	-1.99
GRIP1	Glutamate Receptor Interacting Protein 1	0.006	-1.99
TJP1	Tight Junction Protein 1	0.007	-2.03

MYH15	Myosin Heavy Chain 15	0.040	-2.03
OPCML	Opioid Binding Protein/Cell Adhesion Molecule Like	0.027	-2.04
TM4SF19	Transmembrane 4 L Six Family Member 19	0.002	-2.10
SLC2A12	Solute Carrier Family 2 Member 12	0.046	-2.10
PRSS57	Serine Protease 57	< 0.001	-2.10
TGFB2	Transforming Growth Factor Beta 2	0.019	-2.10
NUAK1	NUAK Family Kinase 1	0.004	-2.13
PLPP2	Phospholipid Phosphatase 2	0.013	-2.13
TXNDC2	Thioredoxin Domain Containing 2	0.049	-2.15
VSTM2B	V-Set And Transmembrane Domain Containing 2B	0.010	-2.23
LCA5	Lebercilin LCA5	0.016	-2.23
COL4A2	Collagen Type IV Alpha 2 Chain	0.030	-2.24
SCHIP1	Schwannomin Interacting Protein 1	0.046	-2.26
PTPN20	Protein Tyrosine Phosphatase Non-Receptor Type 20	0.020	-2.28
GOLGA8Q	Golgin A8 Family Member Q	0.011	-2.30
HCN4	Hyperpolarization Activated Cyclic Nucleotide Gated Potassium Channel 4	0.020	-2.35
ANKRD53	Ankyrin Repeat Domain 53	0.036	-2.37
GABRA5	Gamma-Aminobutyric Acid Type A Receptor Subunit Alpha5	0.029	-2.37
OR2A7	Olfactory Receptor Family 2 Subfamily A Member 7	0.011	-2.43
TMEM108	Transmembrane Protein 108	0.015	-2.45
TACSTD2	Tumor Associated Calcium Signal Transducer 2	0.002	-2.52
TENM4	Teneurin Transmembrane Protein 4	0.007	-2.52
DNAH11	Dynein Axonemal Heavy Chain 11	0.022	-2.55
BCL6B	BCL6B Transcription Repressor	0.025	-2.56
CCL22	C-C Motif Chemokine Ligand 22	0.025	-2.56
CSMD1	CUB And Sushi Multiple Domains 1	0.006	-2.58
KRT79	Keratin 79	0.044	-2.61
HTR2B	5-Hydroxytryptamine Receptor 2B	0.042	-2.71
IQUB	IQ Motif And Ubiquitin Domain Containing	0.047	-2.72

FBLN1	Fibulin 1	0.008	-2.80
CBLN2	Cerebellin 2 Precursor	0.022	-2.83
PLEKHS1	Pleckstrin Homology Domain Containing S1	0.036	-2.89
HOXA7	Homeobox A7	0.007	-2.90
SHC4	SHC Adaptor Protein 4	0.045	-2.91
DMRTC1	DMRT Like Family C1	0.006	-2.91
GABRD	Gamma-Aminobutyric Acid Type A Receptor Subunit Delta	0.001	-2.92
MAEL	Maelstrom Spermatogenic Transposon Silencer	0.026	-3.13
CASQ1	Calsequestrin 1	0.016	-3.34
SMIM10L2B	Small Integral Membrane Protein 10 Like 2B	0.004	-3.63
DNAAF3	Dynein Axonemal Assembly Factor 3	0.014	-3.79
CFAP46	Cilia And Flagella Associated Protein 46	0.008	-4.01
C4BPA	Complement Component 4 Binding Protein Alpha	< 0.001	-4.50
SLC44A5	Solute Carrier Family 44 Member 5	< 0.001	-5.01
TBC1D3G	TBC1 Domain Family Member 3G	< 0.001	-5579.91
TBC1D3H	TBC1 Domain Family Member 3H	< 0.001	$-10{,}9 \times 10^{3}$

Table 9E. List of 504 genes differently modulated in females HR vs females LR from the RNA-Seq analysis (FC ± |1.2|, p-value < 0.05).

Gene Symbol	Gene Assignment	p-value	Fold-Change
TBC1D3K	TBC1 Domain Family Member 3K	0.020	231.14
BTNL3	Butyrophilin Like 3	0.049	7.17
TACSTD2	Tumor Associated Calcium Signal Transducer 2	< 0.001	6.27
DNAH11	Dynein Axonemal Heavy Chain 11	< 0.001	4.94
PCDHGC4	Protocadherin Gamma Subfamily C, 4	0.025	4.56
MUC12	Mucin 12, Cell Surface Associated	0.044	3.89
IGF1	Insulin Like Growth Factor 1	0.003	3.58
FOXI1	Forkhead Box I1	0.004	3.27
DSG3	Desmoglein 3	0.007	2.93
ITGA8	Integrin Subunit Alpha 8	0.005	2.88
DISP2	Dispatched RND Transporter Family Member 2	0.002	2.70
ZNF695	Zinc Finger Protein 695	0.041	2.70
PTPN14	Protein Tyrosine Phosphatase Non-Receptor Type 14	0.031	2.64
MYO16	Myosin XVI	0.010	2.61
ZNF704	Zinc Finger Protein 704	0.013	2.55
VTN	Vitronectin	0.019	2.53
HSF2BP	Heat Shock Transcription Factor 2 Binding Protein	0.016	2.52
CDK15	Cyclin Dependent Kinase 15	0.015	2.51
TRPC6	Transient Receptor Potential Cation Channel Subfamily C Member 6	0.003	2.48
PRSS41	Serine Protease 41	0.005	2.40
MTRNR2L8	MT-RNR2 Like 8	0.008	2.39
DEPDC1	DEP Domain Containing 1	0.015	2.38
TMEM108	Transmembrane Protein 108	0.032	2.36
CCL23	C-C Motif Chemokine Ligand 23	0.025	2.36
LIMCH1	LIM And Calponin Homology Domains 1	0.004	2.34

ABCC11	ATP Binding Cassette Subfamily C Member 11	0.031	2.33
IL36A	Interleukin 36 Alpha	0.009	2.31
C10orf82	Chromosome 10 Open Reading Frame 82	0.043	2.31
OLIG2	Oligodendrocyte Transcription Factor 2	0.002	2.30
PLAAT5	Phospholipase A And Acyltransferase 5	0.001	2.29
ALOX15	Arachidonate 15-Lipoxygenase	0.002	2.29
CCL3L1	C-C Motif Chemokine Ligand 3 Like 1	0.035	2.28
FGFR2	Fibroblast Growth Factor Receptor 2	< 0.001	2.27
PPIAL4C	Peptidylprolyl Isomerase A Like 4C	0.017	2.25
KCNN3	Potassium Calcium-Activated Channel Subfamily N Member 3	0.020	2.24
MROH7	Maestro Heat Like Repeat Family Member 7	0.032	2.24
TNS2	Tensin 2	0.006	2.21
TENT5B	Terminal Nucleotidyltransferase 5B	0.007	2.19
MYCT1	MYC Target 1	0.001	2.18
IDO1	Indoleamine 2,3-Dioxygenase 1	0.001	2.15
SIGLEC8	Sialic Acid Binding Ig Like Lectin 8	0.006	2.13
TNR	Tenascin R	0.004	2.10
UNC5B	Unc-5 Netrin Receptor B	0.011	1.99
PRSS33	Serine Protease 33	0.015	1.99
CCDC40	Coiled-Coil Domain Containing 40	0.043	1.96
CHD5	Chromodomain Helicase DNA Binding Protein 5	0.044	1.96
IL5RA	Interleukin 5 Receptor Subunit Alpha	0.002	1.93
PNMA6A	PNMA Family Member 6A	0.034	1.90
COL4A1	Collagen Type IV Alpha 1 Chain	0.039	1.88
SDC1	Syndecan 1	0.013	1.88
CACNB4	Calcium Voltage-Gated Channel Auxiliary Subunit Beta 4	0.002	1.88
TP73	Tumor Protein P73	0.006	1.88
USP6	Ubiquitin Specific Peptidase 6	0.026	1.87
ADORA3	Adenosine A3 Receptor	0.003	1.84
FRRS1	Ferric Chelate Reductase 1	< 0.001	1.84

SLC16A14	Solute Carrier Family 16 Member 14	0.005	1.84
MYBPH	Myosin Binding Protein H	0.009	1.83
RAG1	Recombination Activating 1	0.033	1.83
ZNF521	Zinc Finger Protein 521	0.035	1.82
SLC29A1	Solute Carrier Family 29 Member 1 (Augustine Blood Group)	0.002	1.81
ITGB8	Integrin Subunit Beta 8	0.016	1.81
ARHGEF39	Rho Guanine Nucleotide Exchange Factor 39	< 0.001	1.81
TRIM36	Tripartite Motif Containing 36	0.001	1.81
PRUNE2	Prune Homolog 2 With BCH Domain	0.035	1.79
BEGAIN	Brain Enriched Guanylate Kinase Associated	0.046	1.78
CCDC141	Coiled-Coil Domain Containing 141	0.007	1.76
DTL	Denticleless E3 Ubiquitin Protein Ligase Homolog	0.025	1.76
CLEC9A	C-Type Lectin Domain Containing 9A	0.003	1.75
CAV2	Caveolin 2	0.028	1.74
CEBPE	CCAAT Enhancer Binding Protein Epsilon	0.002	1.74
MINAR1	Membrane Integral NOTCH2 Associated Receptor 1	0.005	1.74
ERG	ETS Transcription Factor ERG	0.017	1.73
CYP4F12	Cytochrome P450 Family 4 Subfamily F Member 12	0.007	1.73
ACOT11	Acyl-Coa Thioesterase 11	0.004	1.72
LRTOMT	Leucine Rich Transmembrane And O-Methyltransferase Domain Containing	0.031	1.72
TDRD12	Tudor Domain Containing 12	0.036	1.72
ESPNL	Espin Like	0.010	1.72
IL34	Interleukin 34	0.030	1.71
AJAP1	Adherens Junctions Associated Protein 1	0.029	1.71
PTGDR2	Prostaglandin D2 Receptor 2	0.003	1.71
IL1RL1	Interleukin 1 Receptor Like 1	0.030	1.70
BUB1B	BUB1 Mitotic Checkpoint Serine/Threonine Kinase B	0.006	1.70
CCR9	C-C Motif Chemokine Receptor 9	0.002	1.69
SMPD3	Sphingomyelin Phosphodiesterase 3	0.006	1.69

SPNS3	Sphingolipid Transporter 3 (Putative)	0.003	1.68
CCND1	Cyclin D1	0.001	1.68
LGALS12	Galectin 12	0.001	1.67
HRH4	Histamine Receptor H4	0.011	1.67
ESCO2	Establishment Of Sister Chromatid Cohesion N-Acetyltransferase 2	0.025	1.66
SDC2	Syndecan 2	0.025	1.66
RHOBTB3	Rho Related BTB Domain Containing 3	0.001	1.66
ACSM1	Acyl-Coa Synthetase Medium Chain Family Member 1	0.048	1.65
FZD7	Frizzled Class Receptor 7	0.027	1.65
CACNG8	Calcium Voltage-Gated Channel Auxiliary Subunit Gamma 8	0.025	1.65
HDC	Histidine Decarboxylase	0.013	1.64
ASB2	Ankyrin Repeat And SOCS Box Containing 2	0.001	1.63
PRH1	Proline Rich Protein Haeiii Subfamily 1	0.008	1.62
RAB44	RAB44, Member RAS Oncogene Family	0.002	1.62
CXCL6	C-X-C Motif Chemokine Ligand 6	0.030	1.62
CAV1	Caveolin 1	0.023	1.62
FBN1	Fibrillin 1	0.022	1.61
PMP22	Peripheral Myelin Protein 22	0.036	1.60
KIF15	Kinesin Family Member 15	0.036	1.60
ATP2A1	Atpase Sarcoplasmic/Endoplasmic Reticulum Ca2+ Transporting 1	0.026	1.60
POLR3G	RNA Polymerase III Subunit G	0.038	1.59
NPB	Neuropeptide B	0.036	1.58
PARPBP	PARP1 Binding Protein	0.029	1.57
AKAP12	A-Kinase Anchoring Protein 12	0.038	1.56
RHEX	Regulator Of Hemoglobinization And Erythroid Cell Expansion	0.040	1.56
PIK3R6	Phosphoinositide-3-Kinase Regulatory Subunit 6	0.002	1.55
RGL3	Ral Guanine Nucleotide Dissociation Stimulator Like 3	0.046	1.55
CRIP2	Cysteine Rich Protein 2	0.003	1.54

ZC2HC1A	Zinc Finger C2HC-Type Containing 1A	0.021	1.53
SLC18A2	Solute Carrier Family 18 Member A2	0.034	1.53
REXO5	RNA Exonuclease 5	0.010	1.52
IGLL5	Immunoglobulin Lambda Like Polypeptide 5	0.022	1.52
C3	Complement C3	0.037	1.51
DNAJC28	Dnaj Heat Shock Protein Family (Hsp40) Member C28	0.039	1.51
SRGAP3	SLIT-ROBO Rho Gtpase Activating Protein 3	0.026	1.51
MEIS1	Meis Homeobox 1	0.015	1.50
JAKMIP3	Janus Kinase And Microtubule Interacting Protein 3	0.041	1.50
TAS2R14	Taste 2 Receptor Member 14	0.041	1.49
BHLHA15	Basic Helix-Loop-Helix Family Member A15	0.039	1.49
TXNDC5	Thioredoxin Domain Containing 5	0.012	1.48
NT5DC2	5'-Nucleotidase Domain Containing 2	0.002	1.48
ZACN	Zinc Activated Ion Channel	0.024	1.48
ARPIN	Actin Related Protein 2/3 Complex Inhibitor	0.044	1.48
NDFIP2	Nedd4 Family Interacting Protein 2	0.008	1.48
HYAL3	Hyaluronidase 3	0.006	1.47
GAREM2	GRB2 Associated Regulator Of MAPK1 Subtype 2	0.010	1.46
ACE	Angiotensin I Converting Enzyme	0.020	1.46
CD200R1	CD200 Receptor 1	0.001	1.46
USP53	Ubiquitin Specific Peptidase 53	0.006	1.45
TMEM200A	Transmembrane Protein 200A	0.030	1.45
EPN2	Epsin 2	0.004	1.45
ITGA3	Integrin Subunit Alpha 3	0.001	1.43
MZB1	Marginal Zone B And B1 Cell Specific Protein	0.027	1.43
CCN3	Cellular Communication Network Factor 3	0.028	1.42
BUB1	BUB1 Mitotic Checkpoint Serine/Threonine Kinase	0.044	1.42
SLC16A10	Solute Carrier Family 16 Member 10	0.021	1.41
CENPU	Centromere Protein U	0.030	1.40

KLHL14	Kelch Like Family Member 14	0.005	1.40
MED12L	Mediator Complex Subunit 12L	0.027	1.40
ZNF502	Zinc Finger Protein 502	0.006	1.40
ZC3H12C	Zinc Finger CCCH-Type Containing 12C	0.044	1.40
TTC9	Tetratricopeptide Repeat Domain 9	0.001	1.39
ABCB9	ATP Binding Cassette Subfamily B Member 9	0.006	1.38
TRPV3	Transient Receptor Potential Cation Channel Subfamily V Member 3	0.019	1.37
ND6	NADH Dehydrogenase Subunit 6	0.032	1.37
CYSLTR2	Cysteinyl Leukotriene Receptor 2	0.029	1.37
ZNF726	Zinc Finger Protein 726	0.029	1.37
PAPLN	Papilin, Proteoglycan Like Sulfated Glycoprotein	0.034	1.36
GSTM2	Glutathione S-Transferase Mu 2	0.002	1.35
BMPR1A	Bone Morphogenetic Protein Receptor Type 1A	0.012	1.35
CCDC154	Coiled-Coil Domain Containing 154	0.039	1.35
GPHN	Gephyrin	0.009	1.34
TOGARAM 1	TOG Array Regulator Of Axonemal Microtubules 1	0.021	1.34
FAAH	Fatty Acid Amide Hydrolase	0.007	1.33
SEMA7A	Semaphorin 7A (John Milton Hagen Blood Group)	0.008	1.33
CPAMD8	C3 And PZP Like Alpha-2-Macroglobulin Domain Containing 8	0.028	1.32
MEST	Mesoderm Specific Transcript	0.025	1.31
ZCCHC18	Zinc Finger CCHC-Type Containing 18	0.008	1.31
P2RY2	Purinergic Receptor P2Y2	0.040	1.31
CD24	CD24 Molecule	0.024	1.31
ZNF708	Zinc Finger Protein 708	0.030	1.31
L2HGDH	L-2-Hydroxyglutarate Dehydrogenase	0.041	1.30
SEMA6C	Semaphorin 6C	0.032	1.29
PLEKHA7	Pleckstrin Homology Domain Containing A7	0.028	1.29
ARL6IP6	ADP Ribosylation Factor Like Gtpase 6 Interacting Protein 6	0.024	1.28

MAP7	Microtubule Associated Protein 7	0.039	1.27
GIPR	Gastric Inhibitory Polypeptide Receptor	0.043	1.27
ACSF2	Acyl-Coa Synthetase Family Member 2	0.006	1.26
POMT2	Protein O-Mannosyltransferase 2	0.033	1.26
DLEU7	Deleted In Lymphocytic Leukemia 7	0.043	1.26
KCTD3	Potassium Channel Tetramerization Domain Containing 3	0.049	1.25
CHPF	Chondroitin Polymerizing Factor	0.045	1.25
MTAP	Methylthioadenosine Phosphorylase	0.021	1.25
DECR2	2,4-Dienoyl-Coa Reductase 2	0.035	1.25
CDHR3	Cadherin Related Family Member 3	0.047	1.25
ABCB10	ATP Binding Cassette Subfamily B Member 10	0.030	1.24
NUDT17	Nudix Hydrolase 17	0.035	1.23
ITM2C	Integral Membrane Protein 2C	0.041	1.22
IL11RA	Interleukin 11 Receptor Subunit Alpha	0.001	1.22
PDK1	Pyruvate Dehydrogenase Kinase 1	0.008	1.22
GLCCI1	Glucocorticoid Induced 1	0.011	1.22
HIBCH	3-Hydroxyisobutyryl-Coa Hydrolase	0.019	1.22
KLHL3	Kelch Like Family Member 3	0.041	1.21
ABCC1	ATP Binding Cassette Subfamily C Member 1	0.004	1.21
AXIN2	Axin 2	0.024	1.21
IMPACT	Impact RWD Domain Protein	0.021	1.21
CUL9	Cullin 9	0.014	1.21
LNPK	Lunapark, ER Junction Formation Factor	0.035	1.21
RYK	Receptor Like Tyrosine Kinase	0.009	1.20
PROCA1	Protein Interacting With Cyclin A1	0.017	1.20
ANO9	Anoctamin 9	0.022	1.20
HAUS5	HAUS Augmin Like Complex Subunit 5	0.006	1.20
ZNF674	Zinc Finger Protein 674	0.022	1.20
PLOD1	Procollagen-Lysine,2-Oxoglutarate 5-Dioxygenase 1	0.036	-1.20
CEBPB	CCAAT Enhancer Binding Protein Beta	0.045	-1.20

SLC9A8	Solute Carrier Family 9 Member A8	0.030	-1.20
GALK1	Galactokinase 1	0.008	-1.20
MSH5	Muts Homolog 5	0.041	-1.20
ATP6V0C	Atpase H+ Transporting V0 Subunit C	0.013	-1.20
FTH1	Ferritin Heavy Chain 1	0.012	-1.21
NLRP3	NLR Family Pyrin Domain Containing 3	0.013	-1.21
HFE	Homeostatic Iron Regulator	0.038	-1.21
CDKN1A	Cyclin Dependent Kinase Inhibitor 1A	0.028	-1.21
ZBTB16	Zinc Finger And BTB Domain Containing 16	0.040	-1.21
CISH	Cytokine Inducible SH2 Containing Protein	0.012	-1.22
BSG	Basigin (Ok Blood Group)	0.007	-1.22
DDIT4	DNA Damage Inducible Transcript 4	0.015	-1.22
KIF3A	Kinesin Family Member 3A	0.041	-1.22
MAP3K20	Mitogen-Activated Protein Kinase Kinase Kinase 20	0.042	-1.22
SLC2A1	Solute Carrier Family 2 Member 1	0.008	-1.22
IL4R	Interleukin 4 Receptor	0.024	-1.22
B4GALT7	Beta-1,4-Galactosyltransferase 7	0.038	-1.22
SECTM1	Secreted And Transmembrane 1	0.044	-1.22
ASCC2	Activating Signal Cointegrator 1 Complex Subunit 2	0.050	-1.23
PARP11	Poly(ADP-Ribose) Polymerase Family Member 11	0.048	-1.23
SIDT2	SID1 Transmembrane Family Member 2	0.036	-1.23
37469	Argonaute RISC Catalytic Component 2	0.039	-1.23
C1orf198	Chromosome 1 Open Reading Frame 198	0.006	-1.23
EFCAB2	EF-Hand Calcium Binding Domain 2	0.043	-1.23
MFN2	Mitofusin 2	0.023	-1.23
CASP4	Caspase 4	0.008	-1.24
LYPD3	LY6/PLAUR Domain Containing 3	0.047	-1.24
SMCO4	Single-Pass Membrane Protein With Coiled-Coil Domains 4	0.021	-1.24
LILRB2	Leukocyte Immunoglobulin Like Receptor B2	0.013	-1.24
CCDC71L	Coiled-Coil Domain Containing 71 Like	0.033	-1.24

TBC1D8	TBC1 Domain Family Member 8	0.033	-1.24
GBGT1	Globoside Alpha-1,3-N-Acetylgalactosaminyltransferase 1 (FORS Blood Group)	0.020	-1.24
NDUFAF5	NADH:Ubiquinone Oxidoreductase Complex Assembly Factor 5	0.016	-1.24
CSRNP1	Cysteine And Serine Rich Nuclear Protein 1	0.029	-1.24
SIAH2	Siah E3 Ubiquitin Protein Ligase 2	0.002	-1.24
CIR1	Corepressor Interacting With RBPJ, CIR1	0.020	-1.24
MPP1	Membrane Palmitoylated Protein 1	0.019	-1.25
ING2	Inhibitor Of Growth Family Member 2	0.026	-1.25
LILRB4	Leukocyte Immunoglobulin Like Receptor B4	0.022	-1.25
ZNF596	Zinc Finger Protein 596	0.041	-1.25
CHST10	Carbohydrate Sulfotransferase 10	0.015	-1.25
DHRS13	Dehydrogenase/Reductase 13	0.029	-1.25
CARM1	Coactivator Associated Arginine Methyltransferase 1	0.037	-1.25
LGALS3	Galectin 3	0.023	-1.26
NLRC4	NLR Family CARD Domain Containing 4	0.014	-1.26
TUBB6	Tubulin Beta 6 Class V	0.019	-1.26
TREM1	Triggering Receptor Expressed On Myeloid Cells 1	0.049	-1.26
SLC2A3	Solute Carrier Family 2 Member 3	0.048	-1.26
BLVRA	Biliverdin Reductase A	0.041	-1.27
SLC24A4	Solute Carrier Family 24 Member 4	0.040	-1.27
DHRS12	Dehydrogenase/Reductase 12	0.027	-1.27
NOTCH4	Notch Receptor 4	0.038	-1.27
USB1	U6 Snrna Biogenesis Phosphodiesterase 1	0.003	-1.27
SNX8	Sorting Nexin 8	0.010	-1.27
SEMA4A	Semaphorin 4A	0.024	-1.27
CD55	CD55 Molecule (Cromer Blood Group)	0.010	-1.27
PFKFB3	6-Phosphofructo-2-Kinase/Fructose-2,6-Biphosphatase 3	0.039	-1.28
CASP1	Caspase 1	0.016	-1.28
LAPTM4B	Lysosomal Protein Transmembrane 4 Beta	0.027	-1.28

ST3GAL4	ST3 Beta-Galactoside Alpha-2,3-Sialyltransferase 4	0.011	-1.28
FKBP5	FKBP Prolyl Isomerase 5	0.016	-1.28
ZDHHC1	Zinc Finger DHHC-Type Containing 1	0.017	-1.28
FCGR3A	Fc Fragment Of Igg Receptor Iiia	0.026	-1.29
LMNB1	Lamin B1	0.011	-1.29
SIRPD	Signal Regulatory Protein Delta	0.021	-1.29
ME1	Malic Enzyme 1	0.048	-1.29
TECPR2	Tectonin Beta-Propeller Repeat Containing 2	0.034	-1.29
F13A1	Coagulation Factor XIII A Chain	0.036	-1.29
DCAF12	DDB1 And CUL4 Associated Factor 12	0.031	-1.29
ANKH	ANKH Inorganic Pyrophosphate Transport Regulator	0.024	-1.29
NME7	NME/NM23 Family Member 7	0.020	-1.29
LIMS2	LIM Zinc Finger Domain Containing 2	0.041	-1.29
RTN2	Reticulon 2	0.022	-1.29
ZMYND15	Zinc Finger MYND-Type Containing 15	0.034	-1.30
B4GALT5	Beta-1,4-Galactosyltransferase 5	0.017	-1.30
OSM	Oncostatin M	0.022	-1.30
S100A11	S100 Calcium Binding Protein A11	0.011	-1.30
PRDX6	Peroxiredoxin 6	0.012	-1.30
BTF3	Basic Transcription Factor 3	0.046	-1.30
C9orf78	Chromosome 9 Open Reading Frame 78	0.049	-1.30
EMC3	ER Membrane Protein Complex Subunit 3	0.015	-1.30
GABRR2	Gamma-Aminobutyric Acid Type A Receptor Subunit Rho2	0.046	-1.31
GFUS	GDP-L-Fucose Synthase	0.005	-1.31
RNF10	Ring Finger Protein 10	0.006	-1.31
RRP12	Ribosomal RNA Processing 12 Homolog	0.005	-1.31
GRINA	Glutamate Ionotropic Receptor NMDA Type Subunit Associated Protein 1	0.001	-1.32
CDC34	Cell Division Cycle 34, Ubiqiutin Conjugating Enzyme	0.013	-1.32
SLC25A37	Solute Carrier Family 25 Member 37	0.031	-1.32

HAGH	Hydroxyacylglutathione Hydrolase	0.017	-1.32
HLA-DRB5	Major Histocompatibility Complex, Class II, DR Beta 5	0.027	-1.32
SHKBP1	SH3KBP1 Binding Protein 1	0.010	-1.33
RNF208	Ring Finger Protein 208	0.050	-1.33
TBC1D24	TBC1 Domain Family Member 24	0.008	-1.33
ARMCX4	Armadillo Repeat Containing X-Linked 4	0.015	-1.33
SDSL	Serine Dehydratase Like	0.047	-1.34
CYSTM1	Cysteine Rich Transmembrane Module Containing 1	0.012	-1.34
CRAT	Carnitine O-Acetyltransferase	< 0.001	-1.34
CR1	Complement C3b/C4b Receptor 1 (Knops Blood Group)	0.031	-1.34
FCER1G	Fc Fragment Of Ige Receptor Ig	0.007	-1.35
CXCL16	C-X-C Motif Chemokine Ligand 16	0.004	-1.35
IL12RB2	Interleukin 12 Receptor Subunit Beta 2	0.019	-1.35
RBM38	RNA Binding Motif Protein 38	0.004	-1.35
GSPT1	G1 To S Phase Transition 1	0.009	-1.35
RILP	Rab Interacting Lysosomal Protein	0.020	-1.35
TAGLN	Transgelin	0.021	-1.35
RPL36AL	Ribosomal Protein L36a Like	0.034	-1.36
GP1BB	Glycoprotein Ib Platelet Subunit Beta	0.018	-1.36
UBXN6	UBX Domain Protein 6	0.027	-1.36
FKBP8	FKBP Prolyl Isomerase 8	0.035	-1.36
CLU	Clusterin	0.019	-1.37
SPARC	Secreted Protein Acidic And Cysteine Rich	0.013	-1.38
GYPC	Glycophorin C (Gerbich Blood Group)	0.030	-1.38
ZMAT2	Zinc Finger Matrin-Type 2	0.039	-1.38
NFIX	Nuclear Factor I X	0.038	-1.39
NID1	Nidogen 1	0.009	-1.39
TSPAN5	Tetraspanin 5	0.037	-1.39
CST7	Cystatin F	0.011	-1.39
HEMGN	Hemogen	0.025	-1.40

PROK2	Prokineticin 2	0.030	-1.40
CASP5	Caspase 5	0.034	-1.40
SIPA1L2	Signal Induced Proliferation Associated 1 Like 2	0.015	-1.40
AGBL2	AGBL Carboxypeptidase 2	0.007	-1.41
STK32B	Serine/Threonine Kinase 32B	0.023	-1.41
KIR2DL4	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains And Long Cytoplasmic Tail 4	0.038	-1.42
GLRX5	Glutaredoxin 5	0.002	-1.42
ASTL	Astacin Like Metalloendopeptidase	0.039	-1.43
TREML1	Triggering Receptor Expressed On Myeloid Cells Like 1	0.005	-1.43
AIF1	Allograft Inflammatory Factor 1	0.039	-1.43
RGS6	Regulator Of G Protein Signaling 6	0.027	-1.44
FECH	Ferrochelatase	0.005	-1.44
EGR3	Early Growth Response 3	0.022	-1.44
IL18RAP	Interleukin 18 Receptor Accessory Protein	0.041	-1.44
HSPA12A	Heat Shock Protein Family A (Hsp70) Member 12A	0.014	-1.45
EPHB2	EPH Receptor B2	0.029	-1.45
ADM	Adrenomedullin	0.004	-1.45
ALOX15B	Arachidonate 15-Lipoxygenase Type B	0.027	-1.45
NT5M	5',3'-Nucleotidase, Mitochondrial	0.015	-1.45
ADIPOR1	Adiponectin Receptor 1	0.003	-1.45
PIPOX	Pipecolic Acid And Sarcosine Oxidase	0.023	-1.45
SRRD	SRR1 Domain Containing	0.002	-1.45
KLF1	Kruppel Like Factor 1	0.041	-1.46
ZNF480	Zinc Finger Protein 480	0.040	-1.46
ANKRD9	Ankyrin Repeat Domain 9	0.016	-1.46
SOCS3	Suppressor Of Cytokine Signaling 3	0.001	-1.46
CDNF	Cerebral Dopamine Neurotrophic Factor	0.018	-1.47
LRRC37A2	Leucine Rich Repeat Containing 37 Member A2	0.012	-1.47
FHIP1A	FHF Complex Subunit HOOK Interacting Protein 1A	0.009	-1.47

GAS7	Growth Arrest Specific 7	< 0.001	-1.47
KEL	Kell Metallo-Endopeptidase (Kell Blood Group)	0.049	-1.47
DMTN	Dematin Actin Binding Protein	0.026	-1.48
TMEM272	Transmembrane Protein 272	0.014	-1.48
HBM	Hemoglobin Subunit Mu	0.044	-1.48
MXI1	MAX Interactor 1, Dimerization Protein	0.003	-1.48
ZDHHC19	Zinc Finger DHHC-Type Palmitoyltransferase 19	0.040	-1.48
AKR1C3	Aldo-Keto Reductase Family 1 Member C3	0.039	-1.48
PRSS27	Serine Protease 27	0.027	-1.48
ZNF385C	Zinc Finger Protein 385C	0.046	-1.49
GPR146	G Protein-Coupled Receptor 146	0.015	-1.49
KANK2	KN Motif And Ankyrin Repeat Domains 2	0.045	-1.49
SLC25A39	Solute Carrier Family 25 Member 39	0.041	-1.49
ZNF391	Zinc Finger Protein 391	0.030	-1.50
RUNDC3A	RUN Domain Containing 3A	0.026	-1.50
ANXA3	Annexin A3	0.012	-1.50
RAB13	RAB13, Member RAS Oncogene Family	0.001	-1.50
KIR2DL3	Killer Cell Immunoglobulin Like Receptor, Two Ig Domains And Long Cytoplasmic Tail 3	0.023	-1.50
CACNA1E	Calcium Voltage-Gated Channel Subunit Alpha1 E	0.016	-1.50
SAMD14	Sterile Alpha Motif Domain Containing 14	0.050	-1.51
AIM2	Absent In Melanoma 2	0.003	-1.51
BCL2L1	BCL2 Like 1	0.010	-1.51
ST6GALNAC4	ST6 N-Acetylgalactosaminide Alpha-2,6-Sialyltransferase 4	0.002	-1.51
IGF2BP2	Insulin Like Growth Factor 2 Mrna Binding Protein 2	0.005	-1.51
NHSL1	NHS Like 1	0.027	-1.51
TAL1	TAL Bhlh Transcription Factor 1, Erythroid Differentiation Factor	0.031	-1.51
ALPL	Alkaline Phosphatase, Biomineralization Associated	0.018	-1.51
CLEC4D	C-Type Lectin Domain Family 4 Member D	0.031	-1.53

CABP5	Calcium Binding Protein 5	0.021	-1.53
NOTCH2NLC	Notch 2 N-Terminal Like C	0.003	-1.53
OLFML2B	Olfactomedin Like 2B	0.020	-1.54
IFIT1B	Interferon Induced Protein With Tetratricopeptide Repeats 1B	0.024	-1.54
KREMEN1	Kringle Containing Transmembrane Protein 1	0.016	-1.54
CDR2L	Cerebellar Degeneration Related Protein 2 Like	0.031	-1.54
TMEM255B	Transmembrane Protein 255B	0.016	-1.54
MCEMP1	Mast Cell Expressed Membrane Protein 1	0.006	-1.54
DDX11	DEAD/H-Box Helicase 11	0.015	-1.54
TNS1	Tensin 1	0.017	-1.54
KRT73	Keratin 73	0.041	-1.55
CRYM	Crystallin Mu	0.045	-1.56
S100A12	S100 Calcium Binding Protein A12	0.048	-1.56
BBOF1	Basal Body Orientation Factor 1	0.025	-1.57
MYO7B	Myosin VIIB	0.013	-1.57
ITGA2B	Integrin Subunit Alpha 2b	0.004	-1.57
MINDY4	MINDY Lysine 48 Deubiquitinase 4	0.011	-1.58
BPGM	Bisphosphoglycerate Mutase	< 0.001	-1.58
STRADB	STE20 Related Adaptor Beta	0.012	-1.58
ITGB4	Integrin Subunit Beta 4	0.024	-1.59
ALPK3	Alpha Kinase 3	0.003	-1.59
GALNT14	Polypeptide N-Acetylgalactosaminyltransferase 14	0.024	-1.61
TGM2	Transglutaminase 2	0.002	-1.61
TMEM119	Transmembrane Protein 119	0.043	-1.62
TMC4	Transmembrane Channel Like 4	0.013	-1.63
SPATC1	Spermatogenesis And Centriole Associated 1	0.013	-1.63
MARCO	Macrophage Receptor With Collagenous Structure	0.005	-1.64
ALDH1A2	Aldehyde Dehydrogenase 1 Family Member A2	0.009	-1.64

OR2W3	Olfactory Receptor Family 2 Subfamily W Member 3	0.014	-1.64
SH2D4B	SH2 Domain Containing 4B	0.037	-1.65
SPOCD1	SPOC Domain Containing 1	0.005	-1.65
FAM210B	Family With Sequence Similarity 210 Member B	0.002	-1.65
TDRD9	Tudor Domain Containing 9	0.002	-1.65
SLC6A8	Solute Carrier Family 6 Member 8	0.008	-1.66
LILRA5	Leukocyte Immunoglobulin Like Receptor A5	< 0.001	-1.66
SEM1	SEM1 26S Proteasome Subunit	0.015	-1.67
EPB42	Erythrocyte Membrane Protein Band 4.2	0.016	-1.68
C4A	Complement C4A (Rodgers Blood Group)	0.050	-1.68
EMID1	EMI Domain Containing 1	0.005	-1.69
IL27	Interleukin 27	0.006	-1.69
CFD	Complement Factor D	0.012	-1.70
HBQ1	Hemoglobin Subunit Theta 1	0.004	-1.70
SLC6A9	Solute Carrier Family 6 Member 9	0.008	-1.70
SLC41A2	Solute Carrier Family 41 Member 2	0.041	-1.70
PRELID2	PRELI Domain Containing 2	0.040	-1.70
MYL9	Myosin Light Chain 9	0.002	-1.70
ESPN	Espin	0.013	-1.70
SNCA	Synuclein Alpha	0.018	-1.70
HBD	Hemoglobin Subunit Delta	0.021	-1.73
NOTCH2NLB	Notch 2 N-Terminal Like B	0.024	-1.73
WASF3	WASP Family Member 3	0.007	-1.75
TMOD1	Tropomodulin 1	0.006	-1.76
LGALS9B	Galectin 9B	0.003	-1.77
PLEK2	Pleckstrin 2	0.007	-1.78
HSD17B6	Hydroxysteroid 17-Beta Dehydrogenase 6	0.031	-1.78
GMPR	Guanosine Monophosphate Reductase	0.002	-1.79
YBX3	Y-Box Binding Protein 3	< 0.001	-1.81

TSKS	Testis Specific Serine Kinase Substrate	0.016	-1.82
P4HA2	Prolyl 4-Hydroxylase Subunit Alpha 2	0.011	-1.82
KIF26A	Kinesin Family Member 26A	0.014	-1.83
SRGAP1	SLIT-ROBO Rho Gtpase Activating Protein 1	0.043	-1.83
A3GALT2	Alpha 1,3-Galactosyltransferase 2	0.048	-1.83
AHSP	Alpha Hemoglobin Stabilizing Protein	0.005	-1.84
HEPACAM 2	HEPACAM Family Member 2	0.032	-1.85
SPTA1	Spectrin Alpha, Erythrocytic 1	0.025	-1.87
RAMP3	Receptor Activity Modifying Protein 3	0.019	-1.90
TRIM58	Tripartite Motif Containing 58	0.002	-1.90
CLEC2L	C-Type Lectin Domain Family 2 Member L	0.006	-1.91
SYCP3	Synaptonemal Complex Protein 3	0.034	-1.91
SHROOM4	Shroom Family Member 4	0.015	-1.91
VIL1	Villin 1	0.002	-1.92
CRACD	Capping Protein Inhibiting Regulator Of Actin Dynamics	0.027	-1.93
TSPO2	Translocator Protein 2	0.012	-1.94
PPP1R14C	Protein Phosphatase 1 Regulatory Inhibitor Subunit 14C	0.044	-1.97
KRT1	Keratin 1	0.003	-1.97
PF4V1	Platelet Factor 4 Variant 1	0.001	-1.98
LRRC49	Leucine Rich Repeat Containing 49	0.037	-1.98
FAM187A	Family With Sequence Similarity 187 Member A	0.004	-1.99
SLC4A1	Solute Carrier Family 4 Member 1 (Diego Blood Group)	0.004	-2.00
ACHE	Acetylcholinesterase (Cartwright Blood Group)	0.015	-2.01
SIGLEC11	Sialic Acid Binding Ig Like Lectin 11	0.005	-2.06
CA1	Carbonic Anhydrase 1	0.006	-2.07
CARD16	Caspase Recruitment Domain Family Member 16	0.011	-2.08
ODAD4	Outer Dynein Arm Docking Complex Subunit 4	0.005	-2.08
CERS3	Ceramide Synthase 3	0.010	-2.08
SPTB	Spectrin Beta, Erythrocytic	0.001	-2.09

METTL7B	Methyltransferase Like 7B	0.020	-2.10
PRICKLE2	Prickle Planar Cell Polarity Protein 2	0.008	-2.10
PKP3	Plakophilin 3	0.021	-2.10
ADCY10	Adenylate Cyclase 10	0.029	-2.10
SLC6A4	Solute Carrier Family 6 Member 4	0.015	-2.12
NID2	Nidogen 2	0.026	-2.12
MYO10	Myosin X	< 0.001	-2.16
TMC5	Transmembrane Channel Like 5	0.023	-2.16
GGN	Gametogenetin	0.008	-2.18
IFI27	Interferon Alpha Inducible Protein 27	0.027	-2.20
PTGES3L	Prostaglandin E Synthase 3 Like	0.023	-2.22
CYP24A1	Cytochrome P450 Family 24 Subfamily A Member 1	0.037	-2.23
GOLGA8K	Golgin A8 Family Member K	0.011	-2.26
SELENBP1	Selenium Binding Protein 1	< 0.001	-2.32
SMIM6	Small Integral Membrane Protein 6	0.041	-2.35
ALAS2	5'-Aminolevulinate Synthase 2	< 0.001	-2.40
UTS2	Urotensin 2	0.046	-2.40
TMEM132C	Transmembrane Protein 132C	0.009	-2.41
RPH3A	Rabphilin 3A	< 0.001	-2.43
GYPB	Glycophorin B (MNS Blood Group)	0.003	-2.47
SRPX	Sushi Repeat Containing Protein X-Linked	0.011	-2.47
SLC6A19	Solute Carrier Family 6 Member 19	0.050	-2.48
RAP1GAP	RAP1 Gtpase Activating Protein	0.002	-2.51
CD177	CD177 Molecule	0.021	-2.57
TRMT9B	Trna Methyltransferase 9B (Putative)	0.020	-2.58
HMGN5	High Mobility Group Nucleosome Binding Domain 5	0.002	-2.61
SERPINC1	Serpin Family C Member 1	0.006	-2.67
NME1-NME2	NME1-NME2 Readthrough	0.017	-2.72

AIF1L	Allograft Inflammatory Factor 1 Like	0.017	-2.74
SCGB3A1	Secretoglobin Family 3A Member 1	0.014	-2.76
SOX5	SRY-Box Transcription Factor 5	< 0.001	-2.87
ESRRB	Estrogen Related Receptor Beta	0.047	-2.98
GYPA	Glycophorin A (MNS Blood Group)	0.004	-3.00
CSDC2	Cold Shock Domain Containing C2	0.026	-3.12
FAM83A	Family With Sequence Similarity 83 Member A	0.025	-3.34
DET1	DET1 Partner Of COP1 E3 Ubiquitin Ligase	< 0.001	-3.58
EPB41L4B	Erythrocyte Membrane Protein Band 4.1 Like 4B	0.021	-3.62
HBG1	Hemoglobin Subunit Gamma 1	0.022	-4.18
MYOM2	Myomesin 2	< 0.001	-4.36
COX6B2	Cytochrome C Oxidase Subunit 6B2	0.001	-4.74
SUSD5	Sushi Domain Containing 5	< 0.001	-5.02
SMIM34	Small Integral Membrane Protein 34	0.016	-5.60
TBC1D3	TBC1 Domain Family Member 3	< 0.001	-209.06
TBC1D3G	TBC1 Domain Family Member 3G	0.002	-1324.6

APPENDIX F

Table 1F. 33 pathways MDD vs HR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Caveolar-mediated Endocytosis Signaling	< 0.001	0.07		COPE,FLNA,FLOT2,ITGA2B,ITGAM
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.06	2.236	IFIT2,MX1,OAS2,OAS3,STAT2
LPS/IL-1 Mediated Inhibition of RXR Function	< 0.001	0.03	0	ACSL1,CPT1A,IL18RAP,NR1H2,SMOX,TNFRSF1B
Mitochondrial L-carnitine Shuttle Pathway	0.001	0.13		ACSL1,CPT1A
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.003	0.02	2	CYBA,JAK3,MAP3K11,TNFRSF1B
PD-1, PD-L1 cancer immunotherapy pathway	0.004	0.03		CSK,JAK3,TNFRSF1B
Paxillin Signaling	0.005	0.03		CSK,ITGA2B,ITGAM
Interferon Signaling	0.007	0.06		MX1,STAT2
Complement System	0.007	0.06		C4BPA,ITGAM
LXR/RXR Activation	0.007	0.03		IL18RAP,NR1H2,TNFRSF1B
IL-15 Production	0.007	0.03		CSK,JAK3,MAP3K11
Coronavirus Replication Pathway	0.009	0.05		COPE,TUBB1
Melatonin Degradation II	0.010	0.33		SMOX
STAT3 Pathway	0.011	0.02		CSF2RB,IL18RAP,MAP3K11
Spermine and Spermidine Degradation I	0.013	0.25		SMOX
Acetate Conversion to Acetyl-CoA	0.013	0.25		ACSL1
Granulocyte Adhesion and Diapedesis	0.015	0.02		IL18RAP,ITGAM,TNFRSF1B
MSP-RON Signaling Pathway	0.016	0.04		CSF2RB,ITGAM
α-tocopherol Degradation	0.017	0.20		CYP4F3
Activation of IRF by Cytosolic Pattern Recognition Receptors	0.018	0.03		IFIT2,STAT2
Acute Phase Response Signaling	0.022	0.02		C4BPA,HP,TNFRSF1B
PI3K/AKT Signaling	0.023	0.02		CSF2RB,IL18RAP,JAK3
Inositol Pyrophosphates Biosynthesis	0.023	0.14		IP6K1
Natural Killer Cell Signaling	0.026	0.02		IL18RAP,JAK3,MAP3K11
JAK/Stat Signaling	0.030	0.03		JAK3,STAT2
Integrin Signaling	0.031	0.01		ITGA2B,ITGAM,MAP3K11
Osteoarthritis Pathway	0.034	0.01		IL18RAP,NOTCH1,TNFRSF1B
Crosstalk between Dendritic Cells and Natural Killer Cells	0.034	0.02		CSF2RB,TNFRSF1B
Sperm Motility	0.035	0.01		CSK,JAK3,MAP3K11
Fatty Acid Activation	0.043	0.08		ACSL1
Phenylalanine Degradation IV (Mammalian, via Side Chain)	0.043	0.08		SMOX
PPAR Signaling	0.046	0.02		IL18RAP,TNFRSF1B

Oxidative Ethanol Degradation III	0.049	0.07		ACSL1

Table 2F. 9 pathways MDD vs LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	0.002	0.03		IFIT2,MX1
Acute Phase Response Signaling	0.010	0.01		C4BPA,HP
Osteoarthritis Pathway	0.014	0.01		CASP5,IL18RAP
Inflammasome pathway	0.017	0.05		CASP5
Pyrimidine Deoxyribonucleotides De Novo Biosynthesis I	0.018	0.05		CMPK2
Pyrimidine Ribonucleotides Interconversion	0.027	0.03		CMPK2
Pyrimidine Ribonucleotides De Novo Biosynthesis	0.029	0.03		CMPK2
Interferon Signaling	0.032	0.03		MX1
Complement System	0.032	0.03		C4BPA

Table 3F. 11 pathways HR vs LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p.value	Ratio	z-score	Molecules
Melatonin Degradation II	0.001	0.33		SMOX
Spermine and Spermidine Degradation I	0.002	0.25		SMOX
Phenylalanine Degradation IV (Mammalian, via Side Chain)	0.005	0.08		SMOX
Putrescine Degradation III	0.007	0.06		SMOX
Tryptophan Degradation X (Mammalian, via Tryptamine)	0.007	0.06		SMOX
Dopamine Degradation	0.009	0.05		SMOX
Noradrenaline and Adrenaline Degradation	0.012	0.03		SMOX
Serotonin Receptor Signaling	0.017	0.02		SMOX
Serotonin Degradation	0.019	0.02		SMOX
Superpathway of Melatonin Degradation	0.020	0.02		SMOX
Dopamine Receptor Signaling	0.031	0.01		SMOX

Table 4F. 112 pathways males MDD vs males HR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Integrin Signaling	< 0.001	0.10	3.638	ARPC1B,ARPC4,CAPN1,GIT1,ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,MAP3K11,NEDD9,PIK3CD,PLCG2,PXN,RAPGEF1,RHOT2,TLN1,TSPAN3,VASP
Leukocyte Extravasation Signaling	< 0.001	0.10	2.357	ARHGAP1,ARHGAP4,CYBA,ICAM3,ITGAL,ITGAM,ITGB2,MMP25,MSN,NCF2,PIK3CD,PLCG2,PRKCD,PTK2B,PXN,SIPA1,VASP,VAV1
Caveolar-mediated Endocytosis Signaling	< 0.001	0.15		COPE,DNM2,FLNA,FLOT2,HLA-E,ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2
Tec Kinase Signaling	< 0.001	0.09	3.606	GNB2,ITGA5,ITGAL,ITGB2,JAK3,PIK3CD,PLCG2,PRKCD,PTK2B,RHOT2,STAT2,STAT3,STAT5B,STAT6,TYK2,VAV1
HGF Signaling	< 0.001	0.10	3	ELF4,ITGA5,ITGAL,ITGB2,MAP3K11,MAP3K3,PIK3CD,PLCG2,PRKCD,PXN,RAPGEF1,STAT3
TREM1 Signaling	< 0.001	0.13	3	CIITA,ITGA5,ITGAX,LAT2,NLRC3,NLRC5,PLCG2,STAT3,STAT5B
Phagosome Formation	< 0.001	0.10		FCAR,IGHG1,IGHG4,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,PIK3CD,PLCG2,PRKCD,RHOT2
Th1 and Th2 Activation Pathway	< 0.001	0.08		IL4R,IL6R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT3,STAT5B,STAT6,TGFB1,TYK2,VAV1
Fcγ Receptor-mediated Phagocytosis in Macrophages and Monocytes	< 0.001	0.11	3.162	ARPC1B,ARPC4,INPP5D,PLD3,PRKCD,PTK2B,PXN,TLN1,VASP,VAV1
Th2 Pathway	< 0.001	0.09	2.714	IL4R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT5B,STAT6,TGFB1,TYK2,VAV1
IL-8 Signaling	< 0.001	0.08	3.606	CXCR1,GNB2,IKBKE,ITGAM,ITGAX,ITGB2,LASP1,LIMK1,NCF2,PIK3CD,PLD3,PRKCD,PTK2B,RHOT2,VASP
Systemic Lupus Erythematosus Signaling	< 0.001	0.07		CD22,EFTUD2,HLA-E,IGHG1,IGHG4,IL6R,INPP5D,PIK3CD,PIM2,PLCG2,PRPF6,PRPF8,RNU4ATAC,SNRNP200,SNRPA,ZMAT5
Systemic Lupus Erythematosus In B Cell Signaling Pathway	< 0.001	0.07	2.828	CARD11,CD22,IGHA1,IGHG1,IL6R,INPP5D,LILRA6,LTB,MAVS,PIK3CD,PIM2,PLCG2,PRKCD,STAT2,STAT3,TGFB1,TYK2,VAV1
Actin Cytoskeleton Signaling	< 0.001	0.07	3.464	ARPC1B,ARPC4,FGD3,FLNA,GIT1,ITGA5,ITGAL,ITGB2,LIMK1,MSN,MYH9,NCKAP1L,PIK3CD,PXN,TLN1,VAV1

HIF1α Signaling	< 0.001	0.07	2.84	BMP6,GPI,HIF1AN,HK3,HSPA1A/HSPA1B,IL6R,MKNK2,MMP25,NCF2,PIK3CD,PKM,PLCG2,PRKCD,STAT3,TGFB1
Notch Signaling	< 0.001	0.16	0.816	FURIN,MFNG,NOTCH1,NOTCH2,PSENEN,RFNG
Role of JAK1 and JAK3 in γc Cytokine Signaling	< 0.001	0.12		FES,IL4R,JAK3,PIK3CD,PTK2B,STAT3,STAT5B,STAT6
Virus Entry via Endocytic Pathways	< 0.001	0.10		AP1M1,AP2A1,DNM2,FLNA,HLA-E,ITGA5,ITGB2,PIK3CD,PLCG2,PRKCD
Paxillin Signaling	0.001	0.09	2.236	ITGA2B,ITGA5,ITGAL,ITGAM,ITGAX,ITGB2,PIK3CD,PTK2B,PXN,TLN1
Natural Killer Cell Signaling	0.001	0.07	3.742	HLA-E,HSPA1A/HSPA1B,ITGAL,JAK3,LIMK1,MAP3K11,MAP3K3,NCR1,PIK3CD,PLCG2,PTK2B,PXN,TYK2,VAV1
Iron homeostasis signaling pathway	0.001	0.08		ATP6AP1,ATP6V0D1,BMP6,HBD,HBZ,IL6R,JAK3,LRP1,STAT3,STAT5B,TYK2
STAT3 Pathway	0.001	0.08	2.828	BMP6,CSF2RB,CXCR1,IGF2R,IL4R,IL6R,MAP3K11,PIM1,STAT3,TGFB1,TYK2
Th1 Pathway	0.001	0.09	3	IL6R,ITGB2,JAK3,NOTCH1,NOTCH2,PIK3CD,PSENEN,STAT3,TYK2,VAV1
IL-3 Signaling	0.001	0.10	2.121	CSF2RB,INPP5D,PIK3CD,PRKCD,RAPGEF1,STAT3,STAT5B,STAT6
Signaling by Rho Family GTPases	0.001	0.06	3.606	ARHGEF18,ARHGEF2,ARPC1B,ARPC4,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,MSN,NCF2,PIK3CD,PKN1,PTK2B,RHOT2
B Cell Receptor Signaling	0.001	0.07	2.53	BCL6,CD22,IGHA1,IGHG1,IGHG4,IKBKE,INPP5D,MAP3K11,MAP3K3,PIK3CD,PLCG2,PTK2B,VAV1
Rac Signaling	0.002	0.08	2.828	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,NCF2,PIK3CD,PTK2B
Production of Nitric Oxide and Reactive Oxygen Species in Macrophages	0.002	0.07	3.606	CLU,CYBA,IKBKE,JAK3,MAP3K11,MAP3K3,NCF2,PIK3CD,PLCG2,PRKCD,RHOT2,TNFRSF1B,TYK2
RhoGDI Signaling	0.002	0.07	-3.162	ARHGAP1,ARHGAP4,ARHGEF18,ARHGEF2,ARPC1B,ARPC4,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MSN,RHOT2
Ephrin Receptor Signaling	0.002	0.07	2.53	ARPC1B,ARPC4,DOK1,EPHB1,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,PXN,RAPGEF1,SH2D3C,STAT3
T Helper Cell Differentiation	0.003	0.10		BCL6,IL4R,IL6R,STAT3,STAT6,TGFB1,TNFRSF1B
Acute Myeloid Leukemia Signaling	0.003	0.09	0	CSF1R,CSF2RB,CSF3R,PIK3CD,PIM1,PIM2,STAT3,STAT5B

ERK/MAPK Signaling	0.003	0.06	3.317	ELF4,ITGA5,ITGAL,ITGB2,MKNK2,PIK3CD,PLCG2,PRKCD,PTK2B,PXN,RAPGEF1,STAT3,TLN1
PI3K/AKT Signaling	0.004	0.07	1.633	CSF2RB,CXCR1,IKBKE,IL4R,IL6R,INPP5D,ITGA5,ITGAL,ITGB2,JAK3,PIK3CD,TYK2
Phospholipase C Signaling	0.004	0.06	3	ADCY7,ARHGEF18,ARHGEF2,GNB2,HDAC1,IGHG1,IGHG4,ITGA5,ITGAL,ITGB2,ITPR3,PLCG2,PLD3,PRKCD,RHOT2
Acetate Conversion to Acetyl-CoA	0.004	0.50		ACSL1,ACSS1
Role of JAK family kinases in IL-6-type Cytokine Signaling	0.004	0.16		IL6R,STAT3,STAT5B,TYK2
Cdc42 Signaling	0.004	0.07	2.646	ARPC1B,ARPC4,FGD3,HLA-E,ITGA5,ITGAL,ITGB2,LIMK1,MAP3K11,VAV1
Macropinocytosis Signaling	0.004	0.09	2.449	ANKFY1,CSF1R,ITGA5,ITGB2,PIK3CD,PLCG2,PRKCD
Phagosome Maturation	0.005	0.07		ATP6AP1,ATP6V0D1,CTSD,DYNC1H1,GPAA1,HLA-E,NCF2,TUBB1,VPS18,VPS39
Actin Nucleation by ARP-WASP Complex	0.005	0.09	2.236	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,RHOT2,VASP
Reelin Signaling in Neurons	0.005	0.07		ARHGEF2,ARPC1B,ARPC4,ITGA5,LIMK1,MAP3K11,MAPK8IP3,PIK3CD,RAPGEF1
JAK/Stat Signaling	0.006	0.09	2.646	JAK3,PIK3CD,STAT2,STAT3,STAT5B,STAT6,TYK2
SPINK1 General Cancer Pathway	0.006	0.10	2.449	IL6R,JAK3,MT1F,PIK3CD,STAT3,TYK2
Primary Immunodeficiency Signaling	0.006	0.11		CIITA,IGHA1,IGHG1,IGHG4,JAK3
FAK Signaling	0.007	0.08		CAPN1,ITGA5,ITGAL,ITGB2,PIK3CD,PLCG2,PXN,TLN1
Cardiac Hypertrophy Signaling (Enhanced)	0.007	0.05	4.025	ADCY7,ATP2A3,CSF2RB,CXCR1,HDAC1,IKBKE,IL4R,IL6R,ITGA5,ITGAL,ITGB2,ITPR3,LTB,MAP3K11,MAP3K3,MKNK2,PIK3CD,PKN1,PLCG2,PRKCD,STAT3,TGFB1,TNFRSF1B
PAK Signaling	0.008	0.08	1.633	GIT1,ITGA5,ITGAL,ITGB2,LIMK1,PIK3CD,PTK2B,PXN
Gαq Signaling	0.008	0.07	2.333	GNB2,GRK2,IKBKE,ITPR3,PIK3CD,PLCG2,PLD3,PRKCD,PTK2B,RHOT2
Mitochondrial L-carnitine Shuttle Pathway	0.010	0.18		ACSL1,CPT1A,SLC27A3
Semaphorin Neuronal Repulsive Signaling Pathway	0.010	0.07	-0.333	FES,ITGA5,ITGAL,ITGB2,LIMK1,PIK3CD,PLCG2,SEMA4D,STK11
Crosstalk between Dendritic Cells and Natural Killer Cells	0.010	0.08	1.633	CSF2RB,HLA-E,ICAM3,ITGAL,LTB,TLN1,TNFRSF1B
Molecular Mechanisms of Cancer	0.011	0.05		ADCY7,ARHGEF18,ARHGEF2,BAK1,BMP6,CDK9,ITGA5,ITGAL,ITGB2,JAK3,LRP1,NOTCH1,PIK3C

				D,PRKCD,PSENEN,RAPGEF1,RHOT2,TGFB1,TYK2
IL-9 Signaling	0.011	0.12	2	JAK3,PIK3CD,STAT3,STAT5B
Huntington's Disease Signaling	0.012	0.06		CAPN1,CTSD,DCTN1,DNM2,GNB2,GPAA1,HDAC1,HSPA1A/HSPA1B,PIK3CD,POLR2E,POLR2J,POLR2J2/POLR2J3,PRKCD
Insulin Secretion Signaling Pathway	0.013	0.05	3.606	ADCY7,FURIN,GPAA1,ITPR3,JAK3,PIK3CD,PLCG2,PRKCD,STAT2,STAT3,STAT5B,STAT6,TYK2
Purine Nucleotides Degradation II (Aerobic)	0.014	0.16		ADA2,ADAT3,IMPDH1
Inositol Pyrophosphates Biosynthesis	0.014	0.29		IP6K1,PPIP5K1
Adenine and Adenosine Salvage III	0.014	0.29		ADA2,ADAT3
Purine Ribonucleosides Degradation to Ribose-1-phosphate	0.014	0.29		ADA2,ADAT3
Regulation of Cellular Mechanics by Calpain Protease	0.014	0.08		CAPN1,ITGA5,ITGAL,ITGB2,PXN,TLN1
IL-7 Signaling Pathway	0.014	0.08	2	BAK1,BCL6,IGHG1,JAK3,PIK3CD,STAT5B
Mitochondrial Dysfunction	0.014	0.06		ATP5PD,COX6A1,CPT1A,FURIN,NDUFA2,NDUFB1,NDUFB6,OGDH,PSENEN,RHOT2
Clathrin-mediated Endocytosis Signaling	0.015	0.06		AP1M1,AP2A1,ARPC1B,ARPC4,CLU,DNM2,GAK,HGS,ITGA5,ITGB2,PIK3CD
IL-15 Signaling	0.016	0.08	2.449	JAK3,PIK3CD,STAT3,STAT5B,STAT6,TYK2
Regulation of Actin-based Motility by Rho	0.016	0.07	2.236	ARPC1B,ARPC4,ITGA5,ITGAL,ITGB2,LIMK1,RHOT2
Unfolded protein response	0.016	0.09		CD82,ERN1,HSPA1A/HSPA1B,OS9,VCP
Colorectal Cancer Metastasis Signaling	0.017	0.05	3	ADCY7,GNB2,GRK2,IL6R,JAK3,LRP1,MMP25,PIK3CD,PTGER4,RHOT2,STAT3,TGFB1,TYK2
T Cell Exhaustion Signaling Pathway	0.017	0.06	1	BCL6,HLA-E,IL6R,JAK3,PIK3CD,PLCG2,STAT2,STAT3,TGFB1,TYK2
RhoA Signaling	0.017	0.07	1.414	ARHGAP1,ARHGAP4,ARPC1B,ARPC4,LIMK1,MSN,PKN1,PTK2B
Semaphorin Signaling in Neurons	0.019	0.09		ARHGAP1,FES,LIMK1,RHOT2,SEMA4D
FLT3 Signaling in Hematopoietic Progenitor Cells	0.020	0.08	1.633	INPP5D,PIK3CD,STAT2,STAT3,STAT5B,STAT6
Neuregulin Signaling	0.021	0.07	1	ITGA5,ITGAL,ITGB2,PLCG2,PRKCD,RNF41,STAT5B
PD-1, PD-L1 cancer immunotherapy pathway	0.021	0.07	-0.816	HLA-E,JAK3,PIK3CD,STAT5B,TGFB1,TNFRSF1B,TYK2
IL-4 Signaling	0.023	0.07		IL4R,INPP5D,JAK3,PIK3CD,STAT6,TYK2
Triacylglycerol Degradation	0.024	0.10		ABHD2,CES2,PLB1,PNPLA2
IL-22 Signaling	0.026	0.13		STAT3,STAT5B,TYK2

Role of JAK1, JAK2 and TYK2 in Interferon Signaling	0.026	0.13		STAT2,STAT3,TYK2
Glycolysis I	0.026	0.13		ENO1,GPI,PKM
Pancreatic Adenocarcinoma Signaling	0.027	0.06	2.449	JAK3,NOTCH1,PIK3CD,PLD3,STAT3,TGFB1,TYK2
Acetyl-CoA Biosynthesis III (from Citrate)	0.027	1.00		ACLY
Thrombopoietin Signaling	0.028	0.08	2.236	PIK3CD,PLCG2,PRKCD,STAT3,STAT5B
Oncostatin M Signaling	0.028	0.09	2	JAK3,STAT3,STAT5B,TYK2
Calcium Transport I	0.028	0.20		ANXA5,ATP2A3
PDGF Signaling	0.029	0.07	2.449	INPP5D,JAK3,PIK3CD,PLCG2,STAT3,TYK2
PI3K Signaling in B Lymphocytes	0.029	0.06	2.121	ATF6B,IKBKE,IL4R,INPP5D,ITPR3,PIK3CD,PLCG2,VAV1
ErbB2-ErbB3 Signaling	0.029	0.08	2.236	JAK3,PIK3CD,STAT3,STAT5B,TYK2
MSP-RON Signaling In Macrophages Pathway	0.030	0.06	1.134	CIITA,IKBKE,ITGAM,ITGB2,PIK3CD,SBNO2,STAT3
Sperm Motility	0.030	0.05	2.449	CSF1R,EPHB1,FES,ITPR3,JAK3,MAP3K11,PLB1,PLCG2,PRKCD,PTK2B,TYK2
IL-17A Signaling in Airway Cells	0.031	0.08	2.236	IKBKE,JAK3,PIK3CD,STAT3,TYK2
Pyridoxal 5'-phosphate Salvage Pathway	0.031	0.08	2	GRK6,LIMK1,PIM1,PKN1,PRKCD
Remodeling of Epithelial Adherens Junctions	0.032	0.08		ARPC1B,ARPC4,DNM2,HGS,TUBB1
Erythropoietin Signaling Pathway	0.034	0.05	0.333	CSF2RB,HBD,HBZ,ITPR3,LTB,PIK3CD,PRKCD,STAT5B,TGFB1
Axonal Guidance Signaling	0.035	0.04		ARPC1B,ARPC4,BMP6,EPHB1,FES,GIT1,GNB2,ITGA5,ITGAL,ITGB2,LIMK1,MMP25,NTNG2,PIK3CD,PLCG2,PRKCD,PXN,SEMA4D,TUBB1,VASP
FAT10 Cancer Signaling Pathway	0.035	0.09	2	IKBKE,STAT3,TGFB1,TNFRSF1B
PFKFB4 Signaling Pathway	0.035	0.09	1	GPI,HK3,TGFB1,TKT
fMLP Signaling in Neutrophils	0.036	0.06	2.449	ARPC1B,ARPC4,GNB2,ITPR3,NCF2,PIK3CD,PRKCD
Tight Junction Signaling	0.037	0.05		ARHGEF2,CPSF1,F2RL2,GPAA1,MYH9,SYMPK,TGFB1,TNFRSF1B,VASP
CD28 Signaling in T Helper Cells	0.038	0.06	2.646	ARPC1B,ARPC4,CARD11,IKBKE,ITPR3,PIK3CD,VAV1
Renin-Angiotensin Signaling	0.039	0.06	2.236	ADCY7,ITPR3,PIK3CD,PLCG2,PRKCD,PTK2B,STAT3
Synaptogenesis Signaling Pathway	0.040	0.05	3.606	ADCY7,AP2A1,ARPC1B,ARPC4,EPHB1,GPAA1,LIMK1,LRP1,PIK3CD,PLCG2,PRKCD,RAPGEF1,SGTA,TLN1
Spliceosomal Cycle	0.040	0.08	2	EFTUD2,SNRNP200,U2AF2,XAB2
Stearate Biosynthesis I (Animals)	0.040	0.08	2	ACOT8,ACSL1,ELOVL1,SLC27A3
UDP-N-acetyl-D-galactosamine Biosynthesis II	0.040	0.17		GPI,HK3

GM-CSF Signaling	0.041	0.07	2	CSF2RB,PIK3CD,PIM1,STAT3,STAT5B
IL-15 Production	0.041	0.06	2.646	CSF1R,EPHB1,FES,JAK3,MAP3K11,PTK2B,TYK2
Growth Hormone Signaling	0.043	0.07	2.236	PIK3CD,PLCG2,PRKCD,STAT3,STAT5B
Ephrin B Signaling	0.045	0.07	2	EPHB1,GNB2,LIMK1,PXN,VAV1
Assembly of RNA Polymerase II Complex	0.046	0.08	1	POLR2E,POLR2J,POLR2J2/POLR2J3,TAF6
Salvage Pathways of Pyrimidine Ribonucleotides	0.046	0.06	2.236	APOBEC3A,GRK6,LIMK1,PIM1,PKN1,PRKCD
Fatty Acid Activation	0.047	0.15		ACSL1,SLC27A3
Dendritic Cell Maturation	0.047	0.05	2.828	HLA-E,IGHG1,IGHG4,IKBKE,LTB,PIK3CD,PLCG2,STAT2,TNFRSF1B

Table 5F. 28 pathways males MDD vs males LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Primary Immunodeficiency Signaling	< 0.001	0.09		IGHA1,IGHG1,IGHG4,JAK3
Phagosome Formation	< 0.001	0.04		FCAR,IGHG1,IGHG4,ITGAX,PLCG2
TREM1 Signaling	< 0.001	0.06	2	ITGAX,NLRC3,NLRC5,PLCG2
Hematopoiesis from Pluripotent Stem Cells	0.001	0.07		IGHA1,IGHG1,IGHG4
Systemic Lupus Erythematosus Signaling	0.005	0.02		IGHG1,IGHG4,PLCG2,RNU4ATAC,SNRPN
Caveolar-mediated Endocytosis Signaling	0.006	0.04		COPE,FLNA,ITGAX
IL-7 Signaling Pathway	0.006	0.04		BAK1,IGHG1,JAK3
Communication between Innate and Adaptive Immune Cells	0.010	0.03		IGHA1,IGHG1,IGHG4
Dendritic Cell Maturation	0.011	0.02	2	IGHG1,IGHG4,PLCG2,STAT2
B Cell Receptor Signaling	0.013	0.02		IGHA1,IGHG1,IGHG4,PLCG2
Interferon Signaling	0.014	0.06		BAK1,STAT2
Notch Signaling	0.015	0.05		NOTCH1,RFNG
Natural Killer Cell Signaling	0.015	0.02	1	IL18RAP,JAK3,LILRB1,PLCG2
Autoimmune Thyroid Disease Signaling	0.019	0.05		IGHG1,IGHG4
Coronavirus Replication Pathway	0.019	0.05		COPE,TUBB2A
Estrogen Receptor Signaling	0.023	0.02	2	HSP90B1,JAK3,MMP8,NOTCH1,PLCG2
Allograft Rejection Signaling	0.024	0.04		IGHG1,IGHG4
Glucocorticoid Receptor Signaling	0.027	0.01		HP,HSP90B1,IL18RAP,JAK3,MMP8,POLR2J
Pentose Phosphate Pathway (Non-oxidative Branch)	0.030	0.17		TKT
Insulin Secretion Signaling Pathway	0.032	0.02	2	ITPR3,JAK3,PLCG2,STAT2
Inositol Pyrophosphates Biosynthesis	0.035	0.14		IP6K1
Phospholipase C Signaling	0.040	0.02		IGHG1,IGHG4,ITPR3,PLCG2
Salvage Pathways of Pyrimidine Deoxyribonucleotides	0.040	0.13		APOBEC3A
eNOS Signaling	0.041	0.02		HSP90B1,ITPR3,PLCG2
Gαq Signaling	0.041	0.02		GRK2,ITPR3,PLCG2
Aldosterone Signaling in Epithelial Cells	0.043	0.02		HSP90B1,ITPR3,PLCG2
Systemic Lupus Erythematosus In B Cell Signaling Pathway	0.045	0.02	2	IGHA1,IGHG1,PLCG2,STAT2
Pentose Phosphate Pathway	0.049	0.10		TKT

Table 6F. 3 pathways males HR vs males LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
CD27 Signaling in Lymphocytes	0.027	0.02		MAP3K11
Mitotic Roles of Polo-Like Kinase	0.033	0.02		ANAPC1
RANK Signaling in Osteoclasts	0.045	0.01		MAP3K11

Table 7F. 14 pathways females MDD vs females HR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.05	2	CXCL8,IFIT2,MX1,STAT2
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.001	0.05		DHX58,IFIT2,STAT2
Interferon Signaling	0.001	0.06		MX1,STAT2
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza	0.005	0.03		CXCL8,PLAAT5
Systemic Lupus Erythematosus In B Cell Signaling Pathway	0.007	0.01		CXCL8,IFIT2,STAT2
Glycerol Degradation I	0.007	0.20		GK
Atherosclerosis Signaling	0.014	0.02		CXCL8,PLAAT5
IL-6 Signaling	0.015	0.02		CXCL8,TNFAIP6
Coronavirus Pathogenesis Pathway	0.020	0.01		CXCL8,STAT2
Role of IL-17A in Psoriasis	0.021	0.07		CXCL8
Acute Phase Response Signaling	0.030	0.01		C4BPA,HP
Role of JAK1, JAK2 and TYK2 in Interferon Signaling	0.035	0.04		STAT2
IL-17A Signaling in Gastric Cells	0.038	0.04		CXCL8
Airway Inflammation in Asthma	0.047	0.03		CXCL8

Table 8F. 12 pathways females MDD vs females LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.06	2.236	DDX58,EIF2AK2,IFIT2,MX1,RSAD2
Interferon Signaling	< 0.001	0.08		IFIT1,IFITM3,MX1
Role of PKR in Interferon Induction and Antiviral Response	< 0.001	0.03		CASP5,DDX58,EIF2AK2,IFIH1
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.001	0.03	2	DDX58,EIF2AK2,IFIH1,TLR2
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.001	0.05		DDX58,IFIH1,IFIT2
Inflammasome pathway	0.001	0.10		AIM2,CASP5
Role of RIG1-like Receptors in Antiviral Innate Immunity	0.005	0.05		DDX58,IFIH1
TREM1 Signaling	0.013	0.03		CASP5,TLR2
Toll-like Receptor Signaling	0.014	0.03		EIF2AK2,TLR2
Salvage Pathways of Pyrimidine Ribonucleotides	0.023	0.02		CMPK2,EIF2AK2
Phagosome Maturation	0.047	0.01		CTSW,NAPG
Role of Lipids/Lipid Rafts in the Pathogenesis of Influenza	0.048	0.05		RSAD2

Table 9F. 17 pathways females HR vs females LR from microarray analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Pyridoxal 5'-phosphate Salvage Pathway	0.004	0.03		MAP2K3,PIM1
Spermine and Spermidine Degradation I	0.006	0.25		SMOX
Melatonin Degradation II	0.006	0.25		SMOX
dTMP De Novo Biosynthesis	0.008	0.20		DHFR
Xenobiotic Metabolism Signaling	0.008	0.01		FMO4,MAP2K3,SMOX
Acute Myeloid Leukemia Signaling	0.008	0.02		MAP2K3,PIM1
Salvage Pathways of Pyrimidine Ribonucleotides	0.010	0.02		MAP2K3,PIM1
Phenylalanine Degradation IV (Mammalian, via Side Chain)	0.021	0.07		SMOX
Putrescine Degradation III	0.026	0.06		SMOX
Xenobiotic Metabolism CAR Signaling Pathway	0.028	0.01		FMO4,MAP2K3
Tryptophan Degradation X (Mammalian, via Tryptamine)	0.029	0.05		SMOX
Natural Killer Cell Signaling	0.035	0.01		KIR3DL2,MAP2K3
Dopamine Degradation	0.035	0.04		SMOX
HIF1α Signaling	0.038	0.01		MAP2K3,MMP8
LPS/IL-1 Mediated Inhibition of RXR Function	0.042	0.01		FMO4,SMOX
Role of Osteoblasts, Osteoclasts and Chondrocytes in Rheumatoid Arthritis	0.043	0.01		MAP2K3,MMP8
Noradrenaline and Adrenaline Degradation	0.046	0.03		SMOX

APPENDIX G

Table 1G. 19 pathways MDD vs HR from RNA-Seq analysis(p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.18	3.464	CCL2,CXCL10,EIF2AK2,IFIT2,IFIT3,ISG15,MX1,OAS1,OAS2,OAS3,RSAD2,TLR3
Interferon Signaling	< 0.001	0.20	2.646	IFI6,IFIT1,IFIT3,IFITM3,ISG15,MX1,OAS1
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.001	0.08	2.449	C1QA,C1QB,C1QC,EDA,EIF2AK2,IFIH1,OAS1,OAS2,OAS3,TLR3,TNFSF10
Complement System	0.001	0.13	2	C1QA,C1QB,C1QC,C4BPA
Agranulocyte Adhesion and Diapedesis	0.001	0.05		CCL2,CCL25,CCL8,CLDN12,CXCL10,GNAI1,MMP23B,MMP8,MYH11
Granulocyte Adhesion and Diapedesis	0.004	0.05		CCL2,CCL25,CCL8,CLDN12,CXCL10,GNAI1,MMP23B,MMP8
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza	0.005	0.07	0.447	CCL2,CXCL10,EIF2AK2,PLA2G2D,PLA2G4C
Estrogen-mediated S-phase Entry	0.007	0.12		CCNA1,CCNE2,CDC25A
Atherosclerosis Signaling	0.010	0.05		CCL2,COL1A2,LPL,MSR1,PLA2G2D,PLA2G4C
Calcium Transport I	0.010	0.20		ATP2A1,ATP2B3
Activation of IRF by Cytosolic Pattern Recognition Receptors	0.011	0.07	0	DHX58,IFIH1,IFIT2,ISG15
Role of PKR in Interferon Induction and Antiviral Response	0.013	0.05		COLEC12,EIF2AK2,IFIH1,MARCO,MSR1,TLR3
Threonine Degradation II	0.015	1.00		GCAT
Hepatic Fibrosis / Hepatic Stellate Cell Activation	0.018	0.04		BAMBI,CCL2,COL15A1,COL1A2,COL4A4,MYH11,SERPINE1
Coronavirus Pathogenesis Pathway	0.024	0.04	-1.134	CCL2,CCNE2,OAS1,OAS2,OAS3,SERPINE1,TLR3
Role of MAPK Signaling in the Pathogenesis of Influenza	0.030	0.05		CCL2,CXCL10,PLA2G2D,PLA2G4C
Oxytocin Signaling Pathway	0.032	0.03	0.378	ATP2B3,GNAI1,KCNJ8,LPL,MYH11,PLA2G2D,PLA2G4C,PPARG
Glutathione-mediated Detoxification	0.041	0.10		GSTA4,GSTM5
Acute Phase Response Signaling	0.048	0.04		C1QA,C1QB,C1QC,C4BPA,HP,SERPINE1

Table 2G. 44 pathways MDD vs LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.18	3.464	CCL2,DDX58,EIF2AK2,IFIT2,IFIT3,IL1RN,IRF7,ISG15,MX1,OAS2,OAS3,RSAD2
Calcium Signaling	< 0.001	0.08	-2.333	ACTC1,ATP2B2,CAMK2A,CASQ1,CHRNB2,GRIA1,GRIA2,GRIA3,GRIN1,MYH10,MYH11,RYR1,TP63
Glutamate Receptor Signaling	< 0.001	0.13	-2	GRIA1,GRIA2,GRIA3,GRIN1,GRM6,HOMER1,SLC1A2
Neurovascular Coupling Signaling Pathway	< 0.001	0.07	-1.941	GABRD,GABRR2,GAD1,GRIA1,GRIA2,GRIA3,GRIN1,KCNJ10,KCNJ2,KCNMA1,NPR1,RYR1,SLC1A2
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.001	0.08	2.646	C1QB,C1QC,DDX58,EDA,EIF2AK2,IFIH1,IRF7,OAS2,OAS3,TLR5,TNFSF10
Synaptogenesis Signaling Pathway	< 0.001	0.06	-1.807	ADCY2,AFDN,CAMK2A,EFNB2,EPHA5,EPHA7,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,RASD2,STXBP1,STXBP4,STXBP6,WASF1
Gap Junction Signaling	0.001	0.07		ACTC1,ACTG2,ADCY2,GAD1,GRIA1,GRIA2,GRIA3,HTR2B,NPR1,RASD2,TJP1,TUBB3
Interferon Signaling	0.001	0.14	2.236	IFI6,IFIT1,IFIT3,ISG15,MX1
Activation of IRF by Cytosolic Pattern Recognition Receptors	0.002	0.11	1.633	DDX58,IFIH1,IFIT2,IRF7,ISG15,ZBP1
Agrin Interactions at Neuromuscular Junction	0.002	0.10	-1	ACTC1,ACTG2,AGRN,ERBB4,LAMA2,RASD2
Synaptic Long Term Depression	0.003	0.06	-2.121	CRHR1,GAD1,GRIA1,GRIA2,GRIA3,GRM6,NPR1,PPP2R2C,RASD2,RYR1
Neuropathic Pain Signaling In Dorsal Horn Neurons	0.005	0.08	-2.646	CAMK2A,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,KCNQ2
CREB Signaling in Neurons	0.006	0.04	-1.964	ADCY2,ADGRA3,ADGRG6,ADGRL3,BMP6,CAMK2A,CRHR1,FFAR3,FGFR4,GPR176,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,HCAR2,HCAR3,HTR2B,NTRK3,RASD2,TACR3
Tight Junction Signaling	0.008	0.06		ACTC1,ACTG2,AFDN,MAGI2,MYH10,MYH11,NECTIN2,PPP2R2C,TJP1
SNARE Signaling Pathway	0.009	0.07	-1.890	ADCY2,CAMK2A,MYH10,MYH11,STXBP1,STXBP4,STXBP6
Sertoli Cell-Sertoli Cell Junction Signaling	0.010	0.05		ACTC1,ACTG2,AFDN,ITGA8,ITGA9,MAGI2,NECTIN2,RASD2,TJP1,TUBB3
Ephrin Receptor Signaling	0.011	0.05		EFNB2,EPHA5,EPHA7,GRIN1,ITGA8,ITGA9,PTPN13,RASD2,SDC2,VEGFC
Glutathione-mediated Detoxification	0.011	0.14		GGH,GSTA1,GSTA4

Crosstalk between Dendritic Cells and Natural Killer Cells	0.013	0.07	1	ACTC1,ACTG2,CAMK2A,CD80,NECTIN2,TNFSF10
Cellular Effects of Sildenafil (Viagra)	0.014	0.06		ACTC1,ACTG2,ADCY2,KCNQ2,MYH10,MYH11,NPR1
Salvage Pathways of Pyrimidine Deoxyribonucleotides	0.017	0.22		APOBEC3A,APOBEC3B
Glycine Betaine Degradation	0.017	0.22		SARDH,SDS
Synaptic Long Term Potentiation	0.017	0.06	-2.449	CAMK2A,GRIA1,GRIA2,GRIA3,GRIN1,GRM6,RASD2
Epithelial Adherens Junction Signaling	0.018	0.06	0	AFDN,MAGI1,MAGI2,MYH10,NECTIN2,PPP2R2C,RASD2,WASF1
Adrenomedullin signaling pathway	0.021	0.05	-0.378	ADCY2,ADM,GAD1,IL1RN,KCNQ2,NPR1,PPARG,RASD2,TFAP2A
Regulation of Actin-based Motility by Rho	0.023	0.06	-2	ACTC1,ACTG2,ITGA8,ITGA9,PFN2,WASF1
Glutamate Removal from Folates	0.023	1.00		GGH
Threonine Degradation II	0.023	1.00		GCAT
Airway Pathology in Chronic Obstructive Pulmonary Disease	0.024	0.06		CCL2,EDA,ELANE,LCN2,MMP8,TNFSF10
Amyotrophic Lateral Sclerosis Signaling	0.025	0.06	-0.816	GRIA1,GRIA2,GRIA3,GRIN1,SLC1A2,VEGFC
Gustation Pathway	0.026	0.05	0	ABCC8,ADCY2,ASIC2,GABRD,GABRR2,KCNQ2,LPL,SCN4B
cAMP-mediated signaling	0.026	0.05	0	ADCY2,CAMK2A,CRHR1,FFAR3,GRM6,HCAR2,HCAR3,PDE8B,RGS4,TULP2
Osteoarthritis Pathway	0.029	0.05	0.378	CASP5,CASQ1,DCN,IL18RAP,ITGA8,ITGA9,PPARG,PRG4,SMAD1,VEGFC
Oxytocin In Spinal Neurons Signaling Pathway	0.031	0.10		ABCC8,GAD1,NPR1
Complement System	0.031	0.10		C1QB,C1QC,C4BPA
Breast Cancer Regulation by Stathmin1	0.035	0.04	0	ADGRA3,ADGRG6,ADGRL3,BMP6,CAMK2A,CRHR1,E2F7,FFAR3,GPR176,GRM6,HCAR2,HCAR3,HTR2B,PPP2R2C,RASD2,TACR3,TUBB3,VEGFC
Airway Inflammation in Asthma	0.039	0.09		CCL2,ELANE,RNASE2
Circadian Rhythm Signaling	0.041	0.04		ADCY2,CAMK2A,GAD1,GRIA1,GRIA2,GRIA3,GRIN1,NPR1,RASD2,RYR1
Axonal Guidance Signaling	0.043	0.04		ADAMDEC1,BMP6,BMP8A,EFNB2,EPHA5,EPHA7,ITGA8,ITGA9,MMP8,NTNG2,NTRK3,PFN2,RASD2,SDC2,TUBB3,VEGFC
Agranulocyte Adhesion and Diapedesis	0.044	0.05		ACTC1,ACTG2,CCL2,IL1RN,MMP8,MYH10,MYH11,XCL1
Choline Degradation I	0.046	0.50		CHDH
Glutamate Dependent Acid Resistance	0.046	0.50		GAD1
Dilated Cardiomyopathy Signaling Pathway	0.046	0.05	-0.447	ACTC1,ACTG2,ADCY2,CAMK2A,MYH10,MYH11

PTEN Signaling	0.049	0.05	2	FGFR4,ITGA8,ITGA9,MAGI1,MAGI2,NTRK3,RASD2

Table 3G. 41 pathways HR vs LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Pulmonary Fibrosis Idiopathic Signaling Pathway	< 0.001	0.04	-1.265	ACTG2,AREG,CAV1,COL16A1,FGF9,FGFR4,ITGAV,MMP1,MRAS,PDGFC,PLG
Nitric Oxide Signaling in the Cardiovascular System	0.001	0.06	-1.342	CAV1,GUCY1A1,PDE1A,PDGFC,PRKG2,RYR2
Sertoli Cell-Sertoli Cell Junction Signaling	0.002	0.04		ACTG2,GUCY1A1,ITGAV,MRAS,NECTIN2,PRKG2,TUBB3
Neurovascular Coupling Signaling Pathway	0.002	0.04	-1.134	ENTPD3,GRIN3B,GRM5,GUCY1A1,KCNJ10,PRKG2,RYR2
Paxillin Signaling	0.003	0.05	-2	ACTG2,DOCK1,ITGAV,MRAS,TLN2
Actin Cytoskeleton Signaling	0.003	0.03	-2	ACTG2,DOCK1,FGF9,ITGAV,MRAS,PDGFC,TLN2
Gαs Signaling	0.005	0.04	-1	GLP1R,GUCY1A1,MRAS,PTH1R,RYR2
Cellular Effects of Sildenafil (Viagra)	0.005	0.04		ACTG2,GUCY1A1,KCNQ2,PDE1A,PRKG2
Gap Junction Signaling	0.006	0.03		ACTG2,CAV1,GUCY1A1,MRAS,PRKG2,TUBB3
Axonal Guidance Signaling	0.008	0.02		DOCK1,GLI3,ITGAV,MMP1,MRAS,NFATC4,NTRK2,PDGFC,SRGAP1,TUBB3
Integrin Signaling	0.009	0.03	-2.449	ACTG2,CAV1,DOCK1,ITGAV,MRAS,TLN2
ID1 Signaling Pathway	0.009	0.03	-1.633	CAV1,CHRFAM7A,FGFR4,MRAS,PDGFC,TFAP2A
Neuropathic Pain Signaling In Dorsal Horn Neurons	0.012	0.04	-1	GRIN3B,GRM5,KCNQ2,NTRK2
Cardiac β-adrenergic Signaling	0.015	0.03		GUCY1A1,MRAS,PDE1A,RYR2,SLC8A2
Cysteine Biosynthesis III (mammalia)	0.015	0.10		CBS/CBSL,SUV39H2
Osteoarthritis Pathway	0.015	0.03	-1	GLI3,ITGAV,ITLN1,MMP1,PDGFC,PTH1R
Bladder Cancer Signaling	0.016	0.04		FGF9,MMP1,MRAS,PDGFC
Sperm Motility	0.018	0.03	-2	FGFR4,GUCY1A1,MRAS,NTRK2,PDE1A,PRKG2
Choline Degradation I	0.018	0.50		CHDH
Cysteine Biosynthesis/Homocysteine Degradation	0.018	0.50		CBS/CBSL
Synaptic Long Term Depression	0.020	0.03	-1.342	GRM5,GUCY1A1,MRAS,PRKG2,RYR2
Gustation Pathway	0.020	0.03	0	GLP1R,GUCY1A1,KCNQ2,SCN2A,SCN4B
Regulation Of The Epithelial Mesenchymal Transition By Growth Factors Pathway	0.023	0.03	-2	FGF9,FGFR4,MMP1,MRAS,PDGFC
Circadian Rhythm Signaling	0.025	0.02		GRIN3B,GUCY1A1,MRAS,NTRK2,PRKG2,RYR2
Glioma Invasiveness Signaling	0.026	0.04		ITGAV,MRAS,PLG

Adrenomedullin signaling pathway	0.026	0.03	-2.236	GUCY1A1,KCNQ2,MRAS,PRKG2,TFAP2A
Calcium Signaling	0.027	0.03	0.447	CHRFAM7A,GRIN3B,NFATC4,RYR2,SLC8A2
White Adipose Tissue Browning Pathway	0.028	0.03	-2	FGFR4,GUCY1A1,PLIN1,PRKG2
Caveolar-mediated Endocytosis Signaling	0.028	0.04		ACTG2,CAV1,ITGAV
Oxytocin In Spinal Neurons Signaling Pathway	0.032	0.06		GUCY1A1,PRKG2
Opioid Signaling Pathway	0.034	0.02	-0.447	GRIN3B,GUCY1A1,MRAS,PDE1A,POMC,RYR2
Superpathway of Methionine Degradation	0.035	0.06		CBS/CBSL,SUV39H2
STAT3 Pathway	0.035	0.03		FGFR4,IL9R,MRAS,NTRK2
Semaphorin Neuronal Repulsive Signaling Pathway	0.035	0.03	0	DPYSL4,GUCY1A1,ITGAV,PRKG2
Cardiac Hypertrophy Signaling (Enhanced)	0.040	0.02	-0.378	FGF9,FGFR4,GUCY1A1,IL9R,ITGAV,MRAS,NFATC4,PDE1A,RYR2
PDGF Signaling	0.040	0.04		CAV1,MRAS,PDGFC
Regulation of Cellular Mechanics by Calpain Protease	0.044	0.04		ITGAV,MRAS,TLN2
PTEN Signaling	0.045	0.03		FGFR4,ITGAV,MRAS,NTRK2
Crosstalk between Dendritic Cells and Natural Killer Cells	0.045	0.03		ACTG2,NECTIN2,TLN2
Synaptogenesis Signaling Pathway	0.046	0.02	-0.447	GRIN3B,GRM5,GUCY1A1,MRAS,NTRK2,STXBP4
Human Embryonic Stem Cell Pluripotency	0.050	0.03		FGFR4,MRAS,NTRK2,PDGFC

Table 4G. 10 pathways males MDD vs males HR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Mitotic Roles of Polo-Like Kinase	< 0.001	0.12	1.342	CDC20,CDC25A,ESPL1,KIF11,PKMYT1,PLK1,PPM1J
Kinetochore Metaphase Signaling Pathway	0.001	0.07	0.378	AURKB,BUB1,CDC20,ESPL1,KNL1,PLK1,SKA3
Role of CHK Proteins in Cell Cycle Checkpoint Control	0.009	0.08		CDC25A,CLSPN,PLK1,PPM1J
Asparagine Biosynthesis I	0.015	1.00		ASNS
Intrinsic Prothrombin Activation Pathway	0.017	0.09		COL1A2,COL5A3,KLK1
Choline Degradation I	0.031	0.50		ALDH7A1
Atherosclerosis Signaling	0.035	0.04		COL1A2,COL5A3,LPL,PDGFB,PLA2G2D
Glutathione-mediated Detoxification	0.038	0.10		GSTM5,GSTT2/GSTT2B
Anandamide Degradation	0.046	0.33		NAAA
Ethanol Degradation II	0.049	0.09		ADHFE1,ALDH7A1

Table 5G. 27 pathways males MDD vs males LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Mitotic Roles of Polo-Like Kinase	< 0.001	0.17	1.890	CCNB1,CDC20,CDC25A,CDK1,ESPL1,HSP90B1,KIF11,PKMYT1,PLK1,PPP2R3A
Role of CHK Proteins in Cell Cycle Checkpoint Control	< 0.001	0.17	-1.890	BRCA1,CDC25A,CDK1,CLSPN,E2F2,E2F7,E2F8,PLK1,PPP2R3A
Kinetochore Metaphase Signaling Pathway	< 0.001	0.12	1.508	AURKB,BUB1B,CCNB1,CDC20,CDK1,CENPE,ESPL1,KNL1,MAD1L1,PLK1,ZWINT
Estrogen-mediated S-phase Entry	< 0.001	0.23	2.449	CCNA2,CDC25A,CDK1,E2F2,E2F7,E2F8
Cyclins and Cell Cycle Regulation	< 0.001	0.10	2.828	CCNA2,CCNB1,CDC25A,CDK1,E2F2,E2F7,E2F8,PPP2R3A
Epithelial Adherens Junction Signaling	0.001	0.07	-1.265	AFDN,CTNNA2,MAGI1,MAGI2,MET,MYH10,NECTIN2,NOTCH3,PPP2R3A,WASF1
Cell Cycle: G2/M DNA Damage Checkpoint Regulation	0.003	0.10	-0.447	BRCA1,CCNB1,CDK1,PKMYT1,PLK1
Cell Cycle Regulation by BTG Family Proteins	0.005	0.12		E2F2,E2F7,E2F8,PPP2R3A
DNA damage-induced 14-3-3σ Signaling	0.005	0.17		BRCA1,CCNB1,CDK1
Glutathione-mediated Detoxification	0.008	0.15		GGH,GSTA1,PTGES
ATM Signaling	0.011	0.07	0	BRCA1,CCNB1,CDC25A,CDK1,PPP2R3A,TP73
Cell Cycle: G1/S Checkpoint Regulation	0.012	0.07	-2.236	CDC25A,E2F2,E2F7,E2F8,NRG1
Osteoarthritis Pathway	0.015	0.05	0.378	CTNNA2,DCN,ELF3,FZD7,IL18RAP,ITGA8,JAG1,PPARG,PPARGC1A,VEGFC
Pulmonary Healing Signaling Pathway	0.016	0.05	1	CCNB1,CTRC,ELANE,FZD7,JAG1,MMP8,NOTCH3,PRKD1,VEGFC
Role of BRCA1 in DNA Damage Response	0.019	0.07	-0.447	BRCA1,E2F2,E2F7,E2F8,PLK1
Melatonin Degradation III	0.020	1.00		MPO
Glutamate Removal from Folates	0.020	1.00		GGH
Threonine Degradation II	0.020	1.00		GCAT
Phospholipases	0.028	0.07		GPLD1,LPL,PLA2G12A,PLAAT2
Triacylglycerol Degradation	0.040	0.08		FAAH,LPL,PNPLA4
Inhibition of Matrix Metalloproteases	0.040	0.08		MMP8,SDC1,SDC2
Taurine Biosynthesis	0.041	0.50		CDO1
Glycerol-3-phosphate Shuttle	0.041	0.50		GPD1
Iron homeostasis signaling pathway	0.041	0.05		BMP6,BMP8A,BMP8B,HBA1/HBA2,HP,TFR2
Ubiquinol-10 Biosynthesis (Eukaryotic)	0.042	0.13		COQ3,CYP26B1
p53 Signaling	0.044	0.05	2.236	BRCA1,PERP,PLAGL1,TP63,TP73

Basal Cell Carcinoma Signaling	0.047	0.06		BMP6,BMP8A,BMP8B,FZD7

Table 6G. 8 pathways males HR vs males LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Oxidative Phosphorylation	0.002	0.07	-2.646	ATP5PB,ATP5PF,ATP5PO,COX17, NDUFA1,NDUFS5,UQCRB
Mitochondrial Dysfunction	0.007	0.05		ATP5PB,ATP5PF,ATP5PO,COX17, MT-ND6,NDUFA1,NDUFS5,UQCRB
Methylglyoxal Degradation III	0.019	0.17		PTGR1,PTGR2
Dermatan Sulfate Biosynthesis (Late Stages)	0.030	0.08		HS3ST1,SULT1A3/SULT1A4,UST
Choline Degradation I	0.036	0.50		CHDH
Chondroitin Sulfate Biosynthesis (Late Stages)	0.036	0.08		HS3ST1,SULT1A3/SULT1A4,UST
Role of Cytokines in Mediating Communication between Immune Cells	0.044	0.07		CXCL8,IL12A,IL15
Leukocyte Extravasation Signaling	0.048	0.04	0.816	ARHGAP5,CDH5,CLDN12,MMP24,PRKCI,PTK2,RAPGEF3

Table 7G. 49 pathways females MDD vs females HR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
Role of Hypercytokinemia/hyperchemokinemia in the Pathogenesis of Influenza	< 0.001	0.27	4.243	CCL2,CCL3,CXCL10,CXCL8,DDX58,EIF2AK2,IFIT2,IFIT3,IL1RN,IRF7,ISG15,MX1,OAS1,OAS2,OAS3,RSAD2,STAT2,TLR3
Interferon Signaling	< 0.001	0.26	3	IFI6,IFIT1,IFIT3,IFITM3,ISG15,JAK2,MX1,OAS1,STAT2
Role of Pattern Recognition Receptors in Recognition of Bacteria and Viruses	< 0.001	0.10	2.646	C1QB,CXCL8,DDX58,EIF2AK2,IFIH1,IRF7,OAS1,OAS2,OAS3,OSM,PIK3C2A,TLR3,TNFSF10,TNFSF13B
Systemic Lupus Erythematosus In B Cell Signaling Pathway	< 0.001	0.07	2.668	CD19,CD72,CD79A,CXCL8,FCGR2B,IFIH1,IFIT2,IFIT3,IRF7,ISG15,JAK2,OSM,PIK3C2A,STAT2,TLR3,TNFSF10,TNFSF13B
Agranulocyte Adhesion and Diapedesis	< 0.001	0.08		CCL2,CCL3,CCL8,CCR9,CLDN23,CXCL10,CXCL8,GNAI1,IL1RN,MMP24,MMP8,MYH11,XCL1
Granulocyte Adhesion and Diapedesis	< 0.001	0.07		CCL2,CCL3,CCL8,CCR9,CLDN23,CXCL10,CXCL8,GNAI1,IL1RN,MMP24,MMP8,XCL1
Activation of IRF by Cytosolic Pattern Recognition Receptors	< 0.001	0.13	1.134	DDX58,DHX58,IFIH1,IFIT2,IRF7,ISG15,STAT2
TREM1 Signaling	0.001	0.10	2.646	CASP5,CCL2,CCL3,CXCL8,FCGR2B,JAK2,TLR3
Salvage Pathways of Pyrimidine Deoxyribonucleotides	0.001	0.33		APOBEC3A,APOBEC3B,TYMP
Role of MAPK Signaling in Inhibiting the Pathogenesis of Influenza	0.001	0.10	1.89	CCL2,CXCL10,CXCL8,EIF2AK2,PLA2G4A,PLA2G4C,PLA2G7
Airway Pathology in Chronic Obstructive Pulmonary Disease	0.001	0.08		CCL2,CXCL8,LCN12,LCN2,MMP8,OSM,TNFSF10,TNFSF13B
Role of PKR in Interferon Induction and Antiviral Response	0.001	0.07	1.633	CASP5,DDX58,EIF2AK2,IFIH1,IL24,MARCO,MSR1,STAT2,TLR3
Coronavirus Pathogenesis Pathway	0.001	0.06	-1.508	BST2,CCL2,CXCL8,DDX58,IRF7,OAS1,OAS2,OAS3,SERPINE1,STAT2,TLR3
Atherosclerosis Signaling	0.003	0.07		CCL2,CXCL8,IL1RN,MSR1,PLA2G4A,PLA2G4C,PLA2G7,TPSAB1/TPSB2
Role of Macrophages, Fibroblasts and Endothelial Cells in Rheumatoid Arthritis	0.004	0.05		APC2,CCL2,CXCL8,FZD5,IL17RC,IL1RN,JAK2,LRP6,OSM,PIK3C2A,TLR3,TNFSF13B,WNT10A,WNT5B
Communication between Innate and Adaptive Immune Cells	0.004	0.07		CCL3,CD79A,CXCL10,CXCL8,IL1RN,TLR3,TNFSF13B
IL-17 Signaling	0.005	0.06	3	CCL2,CXCL8,IL17RC,JAK2,LCN2,OSM,PIK3C2A,TNFSF10,TNFSF13B
TR/RXR Activation	0.006	0.07		ATP2A1,HP,PIK3C2A,PPARGC1A,STRBP,THRB
Role of RIG1-like Receptors in Antiviral Innate Immunity	0.007	0.11	1	DDX58,DHX58,IFIH1,IRF7

Crosstalk between Dendritic Cells and Natural Killer Cells	0.008	0.07	2	IL15RA,IL3RA,KIR3DL1,TLN2,TLR3,TNFSF10
Role of NANOG in Mammalian Embryonic Stem Cell Pluripotency	0.009	0.06		APC2,FZD5,JAK2,PIK3C2A,TCL1A,WNT10A,WNT5B
Differential Regulation of Cytokine Production in Intestinal Epithelial Cells by IL-17A and IL-17F	0.011	0.13		CCL2,CCL3,LCN2
Role of Cytokines in Mediating Communication between Immune Cells	0.011	0.09		CXCL8,IL1RN,IL24,IL27
Pathogenesis of Multiple Sclerosis	0.014	0.22		CCL3,CXCL10
Role of IL-17F in Allergic Inflammatory Airway Diseases	0.014	0.09		CCL2,CXCL10,CXCL8,IL17RC
Neuroinflammation Signaling Pathway	0.015	0.04	2.714	CCL2,CCL3,CXCL10,CXCL8,GABRD,IRF7,JAK2,P2RX7,PIK3C2A,PLA2G4A,PLA2G4C,TLR3
IL-17A Signaling in Gastric Cells	0.016	0.12		CXCL10,CXCL8,IL17RC
Retinoate Biosynthesis I	0.017	0.11		ALDH1A1,ALDH8A1,RDH10
Role of MAPK Signaling in the Pathogenesis of Influenza	0.020	0.07		CCL2,CXCL10,PLA2G4A,PLA2G4C,PLA2G7
Acute Phase Response Signaling	0.023	0.05	2.236	C1QB,C1R,C4BPA,HP,IL1RN,JAK2,OSM,SERPINE1
Complement System	0.023	0.10		C1QB,C1R,C4BPA
MSP-RON Signaling Pathway	0.023	0.08		CCL2,IL3RA,JAK2,PIK3C2A
Glucocorticoid Receptor Signaling	0.026	0.03		CCL2,CCL3,CXCL8,ESR1,HP,IL15RA,IL17RC,IL1RN,IL31RA,IL3RA,JAK2,MMP8,PIK3C2A,PLA2G4A,PLA2G4C,POMC,SERPINE1
PCP (Planar Cell Polarity) Pathway	0.028	0.07	1	FZD5,PRICKLE1,WNT10A,WNT5B
Role of IL-17A in Arthritis	0.030	0.07		CCL2,CXCL8,IL17RC,PIK3C2A
Airway Inflammation in Asthma	0.030	0.09		CCL2,CXCL8,OSM
Hepatic Fibrosis Signaling Pathway	0.030	0.04	2.714	APC2,CCL2,CCL3,CXCL8,FZD5,GNAI1,IL1RN,JAK2,LRP6,PIK3C2A,SERPINE1,SUCNR1,WNT10A,WNT5B
Role of IL-17A in Psoriasis	0.032	0.14		CXCL8,IL17RC
Salvage Pathways of Pyrimidine Ribonucleotides	0.034	0.06	1.342	APOBEC3A,APOBEC3B,CMPK2,EIF2AK2,NME4
B Cell Development	0.035	0.09		CD19,CD79A,RAG1
Colorectal Cancer Metastasis Signaling	0.036	0.04	1.414	FZD5,GNAI1,JAK2,LRP6,MMP24,MMP8,PIK3C2A,TLR3,WNT10A,WNT5B
Human Embryonic Stem Cell Pluripotency	0.036	0.05		APC2,FZD5,GNAI1,PIK3C2A,S1PR3,WNT10A,WNT5B
HMGB1 Signaling	0.040	0.05	2	CCL2,CXCL8,OSM,PIK3C2A,SERPINE1,TNFSF10,TNFSF13B
Glycerol-3-phosphate Shuttle	0.041	0.50		GPD2
Role of WNT/GSK-3β Signaling in the Pathogenesis of Influenza	0.043	0.06	1	APC2,FZD5,WNT10A,WNT5B
IL-17A Signaling in Fibroblasts	0.043	0.08		CCL2,IL17RC,LCN2

Wound Healing Signaling Pathway	0.044	0.04	2.333	COL15A1,CXCL8,IL1RN,JAK2,MMP8,OSM,TNFSF10,TNFSF13B,TPSAB1/TPSB2
Cardiac Hypertrophy Signaling (Enhanced)	0.046	0.03	2.138	ATP2A1,CXCL8,FZD5,GNAI1,IL15RA,IL17RC,IL31RA,IL3RA,JAK2,OSM,PDE7B,PIK3C2A,TNFSF10,TNFSF13B,WNT10A,WNT5B
Basal Cell Carcinoma Signaling	0.047	0.06		APC2,FZD5,WNT10A,WNT5B

Table 8G. 37 pathways females MDD vs females LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
FAK Signaling	< 0.001	0.05	0	ADGRA3,ADGRF3,ADGRL3,ADRA1B,BCAR3,CCR9,COL18A1,CSF2RB,CXCR1,CXCR2,ERBB2,FFAR2,FZD5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,IL3RA,ITGA7,ITGA9,ITGAV,LPAR2,MRAS,OXTR,PAK6,PDGFRB,S1PR3,TGFB2
Phagosome Formation	< 0.001	0.05	0.365	ADGRA3,ADGRF3,ADGRL3,ADRA1B,APBB1IP,CCR9,CXCR1,FCGR2A,FFAR2,FZD5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,ITGA7,ITGA9,ITGAV,LIMK2,LPAR2,MARCKS,MRAS,MYD88,MYH10,OXTR,PAK6,S1PR3,TLR2,TTN
CREB Signaling in Neurons	< 0.001	0.05	-0.2	ADCY10,ADCY2,ADGRA3,ADGRF3,ADGRL3,ADRA1B,BMP6,CCR9,CXCR1,FFAR2,FGFR4,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,NTRK2,OXTR,PDGFRB,S1PR3,TGFB2
Human Embryonic Stem Cell Pluripotency	0.001	0.08		BMP6,FGFR4,FZD5,GNAQ,GNG5,INHBA,MRAS,NTRK2,PDGFRB,S1PR3,SMAD1,TGFB2
STAT3 Pathway	0.001	0.08	-0.707	BMP6,CSF2RB,CXCR1,CXCR2,FGFR4,IL1B,IL3RA,MRAS,NTRK2,PDGFRB,TGFB2
Breast Cancer Regulation by Stathmin1	0.001	0.05	1	ADGRA3,ADGRF3,ADGRL3,ADRA1B,BMP6,CCR9,CXCR1,FFAR2,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,OXTR,PPP2R2C,PPP2R5A,S1PR3,TGFB2,TUBB3
G-Protein Coupled Receptor Signaling	0.001	0.05	1.134	ADCY10,ADCY2,ADGRA3,ADGRF3,ADGRL3,ADRA1B,BORCS8-MEF2B,CCR9,CXCR1,CXCR2,FFAR2,FZD5,GNAQ,GNG5,GPR153,GPR27,GPRC5C,HCAR2,HCAR3,HTR2B,LPAR2,MRAS,OXTR,PAK6,PDE7B,S1PR3,TTN,WWTR1
RHOGDI Signaling	0.003	0.07	0.816	CDH4,ESR1,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,LIMK2,MRAS,MYH10,PAK6,RHOB
GABA Receptor Signaling	0.006	0.08		ADCY10,ADCY2,GABRA5,GABRD,GABRR2,GNAQ,GNG5,MRAS
Mitochondrial L-carnitine Shuttle Pathway	0.009	0.18		ACSL1,ACSL6,CPT1B
Glycoaminoglycan-protein Linkage Region Biosynthesis	0.009	0.33		B3GAT1,B4GALT7

Gαs Signaling	0.010	0.07	1.134	ADCY10,ADCY2,ADD2,GNAQ,GNG5,HCAR2,HCAR3,MRAS
Cysteine Biosynthesis III (mammalia)	0.016	0.14		CBS/CBSL,EEF1AKMT3,SUV39H2
Cardiac Hypertrophy Signaling (Enhanced)	0.016	0.04	0.728	ADCY10,ADCY2,ADRA1B,BORCS8-MEF2B,CSF2RB,CXCR1,CXCR2,EDA,FGF9,FGFR4,FZD5,GNAQ,GNG5,IL1B,IL3RA,ITGA7,ITGA9,ITGAV,MRAS,PDE7B,TGFB2
Adrenomedullin signaling pathway	0.017	0.06	0.333	ADCY10,ADCY2,ADM,GNAQ,IL1B,IL1RN,MRAS,SHF,TFAP2E,TTN
Gαi Signaling	0.017	0.06	1.134	ADCY10,ADCY2,CXCR2,GNAQ,GNG5,HCAR2,MRAS,S1PR3
CDK5 Signaling	0.018	0.07	-1.134	ADCY10,ADCY2,LAMC1,MRAS,NTRK2,PPP2R2C,PPP2R5A
PAK Signaling	0.019	0.07	-1	ITGA7,ITGA9,ITGAV,LIMK2,MRAS,PAK6,PDGFRB
Hepatic Cholestasis	0.019	0.06		ABCB1,ADCY10,ADCY2,EDA,ESR1,FGFR4,IL1B,IL1RN,MYD88,TGFB2
Natural Killer Cell Signaling	0.021	0.05	1	COL18A1,FCGR2A,HSPA1A/HSPA1B,HSPA6,KIR3DL2,KLRC2,LIMK2,MRAS,MYD88,PAK6
Sphingosine-1-phosphate Signaling	0.022	0.06	1.134	ADCY10,ADCY2,CASQ1,GNAQ,PDGFRB,RHOB,S1PR3
PI3K/AKT Signaling	0.025	0.05		CSF2RB,CXCR1,CXCR2,IL3RA,ITGA7,ITGA9,ITGAV,MRAS,PPP2R2C,PPP2R5A
Osteoarthritis Pathway	0.026	0.05	1.134	CASQ1,CXCR2,FZD5,IL1B,ITGA7,ITGA9,ITGAV,PTCH1,S1PR3,SMAD1,TLR2
eNOS Signaling	0.030	0.06	-0.378	ADCY10,ADCY2,CCNA1,ESR1,GNAQ,HSPA1A/HSPA1B,HSPA6,LPAR2
IL-1 Signaling	0.030	0.07		ADCY10,ADCY2,GNAQ,GNG5,MRAS,MYD88
Stearate Biosynthesis I (Animals)	0.032	0.09	-1	ACSL1,ACSL6,BDH2,DHCR24
Agranulocyte Adhesion and Diapedesis	0.032	0.05		CCL22,CCR9,CLDN9,CXCL16,CXCR1,CXCR2,IL1B,IL1RN,MYH10
Axonal Guidance Signaling	0.034	0.04		ADAMTS6,BMP6,ERBB2,FZD5,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,LIMK2,MRAS,NTRK2,PAK6,PTCH1,SEMA4G,SHANK2,TUBB3,UNC5B
PPARα/RXRα Activation	0.036	0.05	0.707	ACOX1,ADCY10,ADCY2,CPT1B,GK,GNAQ,IL1B,MRAS,TGFB2
Dopamine Receptor Signaling	0.036	0.07		ADCY10,ADCY2,NCS1,PPP2R2C,PPP2R5A
Phospholipase C Signaling	0.041	0.05	1.89	ADCY10,ADCY2,BORCS8-MEF2B,FCGR2A,GNAQ,GNG5,ITGA7,ITGA9,ITGAV,MARCKS,MRAS,RHOB
Cardiac β-adrenergic Signaling	0.041	0.05	-0.816	ADCY10,ADCY2,GNAQ,GNG5,MRAS,PDE7B,PPP2R2C,PPP2R5A

Fatty Acid Activation	0.043	0.15		ACSL1,ACSL6
Hepatic Fibrosis Signaling Pathway	0.046	0.04	0.905	COL18A1,FTH1,FZD5,IL1B,IL1RN,ITGA7,ITGA9,ITGAV,LRP5,MRAS,MYD88,PDGFRB,PTCH1,RHOB,TGFB2,TTN
Sertoli Cell-Sertoli Cell Junction Signaling	0.047	0.05		ADCY10,CLDN9,ITGA7,ITGA9,ITGAV,MRAS,PALS2,TJP1,TUBB3
Superpathway of Methionine Degradation	0.049	0.09		CBS/CBSL,EEF1AKMT3,SUV39H2
RAC Signaling	0.050	0.05	-1	ITGA7,ITGA9,ITGAV,LIMK2,MCF2L,MRAS,PAK6

Table 9G. 34 pathways females HR vs females LR from RNA-Seq analysis (p-value < 0.05)

Ingenuity Canonical Pathways	p-value	Ratio	z-score	Molecules
STAT3 Pathway	< 0.001	0.10	1.134	BMPR1A,CDKN1A,CISH,FGFR2,IGF1,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,MAP3K20,SOCS3
Inflammasome pathway	< 0.001	0.25	-2.236	AIM2,CASP1,CASP5,NLRC4,NLRP3
Osteoarthritis Pathway	< 0.001	0.07	-0.577	ALPL,ANKH,BMPR1A,CASP1,CASP4,CASP5,CEBPB,DDIT4,FZD7,IL18RAP,IL1RL1,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
PI3K/AKT Signaling	0.001	0.07	1	BCL2L1,CCND1,CDKN1A,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Caveolar-mediated Endocytosis Signaling	0.003	0.10		CAV1,CD55,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Granulocyte Adhesion and Diapedesis	0.005	0.07		CCL23,CCL3L1,CCR9,CXCL16,CXCL6,HRH4,IL18RAP,IL1RL1,IL36A,SDC1,SDC2
PTEN Signaling	0.006	0.07	-1.633	BCL2L1,BMPR1A,CCND1,CDKN1A,FGFR2,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
Eicosanoid Signaling	0.007	0.10		AKR1C3,ALOX15,ALOX15B,CYSLTR2,PLAAT5,PRDX6
Cardiac Hypertrophy Signaling (Enhanced)	0.008	0.05	1.213	ACE,ADCY10,ATP2A1,CACNB4,FGFR2,FZD7,IGF1,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL36A,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MAP3K20,OSM,PDK1,PIK3R6
Complement System	0.009	0.13	1	C3,CD55,CFD,CR1
TREM1 Signaling	0.011	0.09	-1.633	CASP1,CASP5,IL1RL1,NLRC4,NLRP3,TREM1
IL-10 Signaling	0.012	0.09		BLVRA,IL18RAP,IL1RL1,IL36A,IL4R,SOCS3
IL-13 Signaling Pathway	0.014	0.07	0.378	ALOX15,ALOX15B,BCL2L1,CXCL6,IL4R,PIK3R6,SOCS3
ID1 Signaling Pathway	0.016	0.06	0.905	BCL2L1,BHLHA15,BMPR1A,CAV1,CCND1,CDKN1A,FGFR2,GSPT1,PIK3R6,RAP1GAP,TGM2
Iron homeostasis signaling pathway	0.019	0.07		ABCB10,ATP6V0C,BMPR1A,CDC34,FECH,FTH1,HFE,SLC25A37
Phagosome Formation	0.022	0.04	0.816	ADORA3,C3,CCR9,CLEC4D,CR1,CYSLTR2,FCER1G,FCGR3A/FCGR3B,FZD7,GPR146,HRH4,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MARCO,MYO10,P2RY2,PIK3R6,PLAAT5,PRDX6,PTGDR2,VTN
JAK/STAT Signaling	0.023	0.07	0.816	BCL2L1,CDKN1A,CEBPB,CISH,PIK3R6,SOCS3
Glycine Betaine Degradation	0.023	0.22		PIPOX,SDSL

HGF Signaling	0.025	0.06	2	CCND1,CDKN1A,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,PIK3R6
Pyroptosis Signaling Pathway	0.026	0.07	-2.449	AIM2,CASP1,CASP4,CASP5,NLRC4,NLRP3
Regulation of Cellular Mechanics by Calpain Protease	0.027	0.07		CCND1,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8
GDP-L-fucose Biosynthesis I (from GDP-D-mannose)	0.027	1.00		GFUS
Histamine Biosynthesis	0.027	1.00		HDC
Sertoli Cell-Sertoli Cell Junction Signaling	0.028	0.05		ADCY10,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,MAP3K20,SPTA1,TUBB6,YBX3
Th2 Pathway	0.028	0.06	0	HLA-DRB5,IL12RB2,IL1RL1,IL4R,NOTCH4,PIK3R6,PTGDR2,SOCS3
Androgen Biosynthesis	0.029	0.20		AKR1C3,HSD17B6
Type II Diabetes Mellitus Signaling	0.032	0.06		ACSF2,ACSM1,ADIPOR1,CACNB4,CEBPB,PIK3R6,SMPD3,SOCS3
Retinoate Biosynthesis I	0.036	0.11		AKR1C3,ALDH1A2,HSD17B6
FAK Signaling	0.037	0.04	1.877	ADORA3,CCND1,CCR9,CYSLTR2,FCER1G,FZD7,GPR146,HRH4,IL11RA,IL12RB2,IL18RAP,IL1RL1,IL4R,IL5RA,ITGA2B,ITGA3,ITGA8,ITGB4,ITGB8,P2RY2,PIK3R6,PTGDR2,SOCS3
Th1 and Th2 Activation Pathway	0.038	0.05		HLA-DRB5,IL12RB2,IL1RL1,IL27,IL4R,NOTCH4,PIK3R6,PTGDR2,SOCS3
Endocannabinoid Cancer Inhibition Pathway	0.044	0.06	-1.414	ADCY10,CASP1,CASP4,CASP5,CCND1,CDKN1A,PIK3R6,SMPD3
Fatty Acid Activation	0.048	0.15		ACSF2,ACSM1
Colanic Acid Building Blocks Biosynthesis	0.048	0.15		GALK1,GFUS
LPS/IL-1 Mediated Inhibition of RXR Function	0.048	0.05		ABCB9,ACSF2,ACSM1,ALDH1A2,CHST10,CRAT,GSTM2,IL18RAP,IL1RL1,IL36A