Exploring the aetiology of psychological distress and its associations with physical health and health-related quality of life

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Exploring the aetiology of psychological distress and its associations with physical health and health-related quality of life

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PhD Thesis, 2021

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Submitted for the degree of Doctor of Philosophy in Behavioural Genetics

Abstract

Mental and physical health often co-occur, and this comorbidity can negatively impact quality of life. Twin studies suggest heritability of measures of psychological distress, including anxiety and depression, as well as physical health markers such as cardiovascular health. Yet, there is still limited behaviour genetic and epigenetic work in understanding how these two domains are related, as well as lack of representation in non-western populations.

The first empirical project involves investigating the genetic and environmental architecture of anxiety symptoms in a Dutch twin sample (Twins Interdisciplinary Neuroticism Study; TWINS) and exploring its associations with three indicators of cardiac autonomic functioning: inter-beat interval, heart rate variability and baroreflex sensitivity. This multivariate twin project adds to much needed behaviour genetics research on the relationships between anxiety and cardiovascular health and is now published as a research paper in *Twin Research & Human Genetics* (Nas, Riese, van Roon & Rijsdijk, 2020).

The second research project involves bivariate twin analyses between anxiety symptoms and health-related quality of life in a Sri-Lankan twin and singleton population (Colombo Twin and Singleton study; CoTASS). This project explores both the aetiology of these traits, as well as providing insight into their covariance, via phenotypic, genetic, and environmental correlations. This project is published as a research paper in *Behaviour Genetics* (Nas et al, 2021). This CoTASS dataset was also used in my third project, which seeks to answer remaining questions regarding causality between psychological distress and physical wellbeing. Using the direction-of-causation (DOC) twin model, I show a potential causal

direction suggesting that poor mental health may be a precursor to decline in physical health. The paper stemming from this project is currently under review in the *Journal of Affective Disorders Reports*.

The fourth component of this thesis involves investigating the epigenetic nature of psychological distress. Although there has been a growing literature surrounding DNA methylation and its involvement in psychiatric conditions, there is relatively little known about the role of global DNA methylation. In this project, we explore the relationships between global DNA methylation (quantified through the repetitive marker, LINE-1 in DNA) & severity of psychological distress using twin datasets from ongoing studies (Social Relationships Study; SRS & Twins' Early development Study; TEDS). In addition, we make use of the twin nature of the datasets to conduct within-twin analyses to investigate the role of epigenetic profiles in psychological distress. In the final chapter I discuss these findings, in light of possible limitations as well as possible future directions.

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Statement of Authorship

All work presented in this thesis is my own. Data collection for all study samples was completed by research teams prior to my involvement. All investigations were conceived and carried out by me, as first author, in collaboration with colleagues included in the author lists at the start of each chapter. Chapter-specific author contribution summaries are shown below.

Zeynep Nas

Chapter 3. ZN and FR conceived the study. FR and HR were involved in the TWINS study formulation and data pre-processing. ZN performed the twin modelling analysis supervised by FR. ZN and FR wrote the manuscript. AR provided expertise regarding the cardiovascular autonomic measures. All authors contributed to manuscript revision, read, and approved the submitted version.

Chapter 4. ZN and FR conceived the study. FR, HZ, AS, KJ, SS and MH were involved in the COTASS study formulation. ZN performed the twin modelling analysis supervised by FR. ZN and FR wrote the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

Chapter 5. ZN conceived the study and conducted analyses under the supervision of FR. All authors provided critical revision of the manuscript.

Chapter 6. ZN conceived the study and conducted analyses under the supervision of CW and HZ. DNA extraction was completed prior to me beginning the project by data teams. All co-authors will review the manuscript shortly.

Publications resulting from the work in this thesis

Published:

Chapter 3
Nas, Z., Riese, H., Van Roon, A., & Rijsdijk, F. (2020). Higher Anxiety Is Associated with Lower Cardiovascular Autonomic Function in Female Twins. Twin Research and Human Genetics, 23(3), 156-164.

Chapter 4
Nas, Z., Zavos, H., Sumathipala, A., Jayaweera, K., Siribaddana, S., Hotopf, M., & Rijsdijk, F. V. (2021). Associations Between Anxiety Symptoms and Health-Related Quality of Life: A Population-Based Twin Study in Sri Lanka. *Behavior genetics*, *51*(4), 394–404.

In preparation:

Chapter 5

Nas, Z., Zavos, H., Sumathipala, A., Jayaweera, K., Siribaddana, S., Hotopf, M., & Rijsdijk, F. V. Causal links between psychological distress and physical health. (Under review in *Journal of Affective Disorders Reports*, 2021).

Additional publications

Triantafyllou, P., **Nas, Z**., Zavos, H. M., Sumathipala, A., Jayaweera, K., Siribaddana, S. H., ... & Rijsdijk, F. V. (2022). The aetiological relationship between depressive symptoms and health-related quality of life: A population-based twin study in Sri Lanka. Plos one, 17(3), e0265421.

Acknowledgements

First and foremost, I would like to offer a huge thanks to my fabulous supervisors. Thank you to my first supervisor, Chloe Wong for sharing your time, knowledge, and advice. I thoroughly enjoyed our discussions, catchups over food and all the fun you put into science. Special thanks to my second supervisor, Frühling Rijsdijk for providing direction to all my research questions and your patience in teaching me twin modelling. You provided all the reassurance I needed, and your steadiness kept me calm. I am truly grateful for all that I have learnt and feel very lucky to have had such supportive role models.

I am also thankful to have worked with other experts, particularly Helena Zavos for the valuable input in several of my research projects. The work in this thesis would also not be possible without the willingness and time of the thousands of participants, admin teams and researchers of the TWINS, COTASS, TEDS and SRS cohorts.

The SGDP centre has been a wonderful place to study, I am so lucky to have been surrounded by such great minds. Thank you to Cathryn Lewis for her wonderful leadership and support, Cathy Fernandes for all the kind words and Louise Arseneault for being a supportive PhD coordinator. Special thanks to my team members: Olakunle Oginni for always making the time, all the twin modelling scribbles and for being a fab travel buddy; and Kai Lim whom I've had the pleasure of sharing an office with, for all the lunches and sharing your wins and worries with me. Thanks also to Megan Skelton, Georgina Krebs, Natali Bozhilova, Jenny Murphy, Rebecca Green, Marilia Pitharouli and Isabella Vainieri for being so friendly, helpful, and relatable. I'd also like to thank Yasmin Ahmadzadeh and Ziada Ayorech for the encouragement, support and all the big sister advice. I also want to acknowledge the anti-racism working group at the SGDP: thank you for being catalysts for change.

I have been extremely fortunate to have met two amazing friends during my time at the SGDP. Mary Agyapong and Keertana Ganesan, thank you for your sisterhood, all the laughter, our podcast-worthy discussions, pasta dates and for listening to all of my venting. Navigating the academic space as an underrepresented individual is hard, and you really made it easier. Huge thanks also to my friends outside the academic environment for cheering me on, providing space for my sniffles and for all the fun - you know who you are.

Special thanks to my examiners, Claire Haworth and Margherita Malanchini for a stimulating and enjoyable viva. I'll be reflecting on our discussions for a long time.

My family has been my grounding centre throughout my PhD journey. I want to thank my mum for ensuring that my health and wellbeing is always the priority, my dad for encouraging me to do a PhD in the first place and my brother for all the laughter when I needed it. Our gatherings at four pm took me out of daily screen time and gave me a much-needed break. I would also like to acknowledge myself. Completing this thesis took hard work, dedication, and lots of perseverance. It was definitely not an easy journey (especially not during a global pandemic!), and I am thankful for who I've become despite all my hardships and doubts. Finally, praise goes to Allah Almighty for the strength and guidance, always.

List of commonly used abbreviations in this thesis

Abbreviation	Description
A / a²	Additive genetic influences
AIC	Akaike's Information Criterion
ANS	Autonomic Nervous System
BIC	Bayesian Information Criterion
BMI	Body Mass Index
BP	Blood Pressure
BRS	Baroreflex Sensitivity
C / c²	Common / shared environmental influences
COTASS	Colombo Twin and Singleton Study
DNA	Deoxyribonucleic Acid
DZ	Dizygotic
E / e²	Unique environmental influences
GWAS	Genome-Wide Association Study
HRQOL	Health Related Quality of Life
HRV	Heart Rate Variability
IBI	Inter Beat Interval
LINE-1	Long Interspersed Nuclear Element 1
MZ	Monozygotic
PRS	Polygenic risk score
rA	Genetic correlation
rC	Common / shared environmental correlation
rE	Unique environmental correlation
rPh	Phenotypic correlation
rPh-A	Phenotypic correlation due to genetic influences
rPh-C	Phenotypic correlation due to common environmental influences
rPh-E	Phenotypic correlation due to unique environmental influences
SEM	Structural Equation Modelling
SNP	Single Nucleotide Polymorphism
SRS	Social Relationships Study
TEDS	Twin's Early Development Study
TWINS	Twin Interdisciplinary Neuroticism Study

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Chapter 1 . Introduction

Psychological distress has been the focus of research for many decades. Definitions are broad and range from disorders of mood (e.g., anxiety and depression) to psychotic conditions (e.g., schizophrenia and bipolar disorder). This psychiatric perspective, however, is categorical in nature, meaning that they may not be representative of psychological distress experienced in the general population. It is now understood that psychological distress is dimensional and normally distributed in the population, hence continuous measures capturing symptomatology of distress is seen as an advantage in research. It is also now known that psychological distress is interrelated with physical ill health. In this chapter, I introduce the concept and definition of psychological distress in this thesis, which centres around internalising problems. Next, I provide an overview of its epidemiology and present current findings of the aetiology of different internalising symptoms based on data from genetically informative designs. I then move onto what is currently known about the relationship between markers of psychological distress and physical health. To end, I summarise the main research gaps and thesis aims to address outstanding questions in the field.

1.1. Psychological distress

1.1.1 Internalising problems

The terms 'internalising' and 'externalising' are used as broad terms to cover two groups of behavioural, emotional, and social problems. Internalising problems usually refer to an

inwards direction of, and appraisal of problems, such as mood decline and withdrawal whereas externalising problems widely refer to an outwards direction of problems such as aggression and substance use (Garnefski et al., 2005). The focus of psychological distress in this thesis is mainly on internalising problems, which mainly refer to anxiety and depression (Achenbach et al., 2016), but also a range of other traits including somatic symptoms (Rieffe & De Rooij, 2012). These internalising traits often co-occur with other psychological problems including externalising traits such as conduct disorder (Polier et al., 2012), with cognitive processing, biases and negative regulation strategies (Garnefski et al., 2005; Hankin et al., 2016), as well as autonomic dysregulation (Chalmers et al., 2014; Kemp et al., 2010).

1.1.2 Continuous vs Diagnostic approach to psychological distress

The question of whether mental health problems are distinct categories as opposed to a spectrum of functioning is a long-standing debate (Widiger & Samuel, 2005). As with other complex traits, psychological distress is now understood to be normally distributed in the population, with underlying genetic and environmental liability. Research has increasingly adapted to this continuous perspective with its various advantages over a diagnostic model. This shift in thought has arisen from many decades of empirical research increasingly indicating the outdated nature of a diagnostic model. Firstly, both from a research and clinical viewpoint, a continuous model is deemed more inclusive, allowing individuals who may not necessarily meet diagnostic criteria to be included in studies and potentially, in accessing treatment options.

Second, excessive comorbidity in clinical settings between categories of disorders, such as

significant overlap between anxiety and depression, suggests the possibility of shared pathology possibly through a common (e.g., internalising) dimension. Recent efforts to characterise psychiatric conditions provide evidence for this higher order internalising factor encapsulating symptoms of psychological distress (Caspi et al., 2014). A meta-analysis of taxometric research (briefly, research that aims to decide between categorical or dimensional models) also indicated that differences between individuals in complex traits such as psychopathology are those of degree, not kind (Haslam et al., 2020). Dimensional findings, especially in relation to anxiety, was overwhelming in comparison to taxonic, categorical findings.

Categorical approaches, however, are still a familiar and predominantly used approach in clinical settings. It has also provided advantages in terms of providing answers to patients (by assigning a diagnosis), as well as allowing communication between clinicians and has been useful for teaching purposes (First et al., 2018). In attempt to conserve these advantages and support empirical data, research has developed working models. One such example is the Hierarchical Taxonomy of Psychopathology (HiTOP) (Kotov et al., 2017). One of the aims of HiTOP is to translate available data on the dimensions of mental health problems into an evidence-based diagnostic classification.

Building on the 'p' factor concept, this model encapsulates a spectrum of symptoms, within a hierarchy of dimensional syndromes (e.g., internalising problems). Designed for both research and clinical practice, its future application can unite both worlds, providing hope for a more inclusive and research-backed notion of mental health (Conway & Krueger, 2021). It is important to note, however, that although the underlying distribution/liability to

psychological distress may be dimensional and normally distributed, measures of these symptoms may still produce skewed distributions.

In this next section, I discuss in more detail what is currently known of the three broad categories of internalising problems covering anxiety, depression, and somatisation.

1.2. Anxiety

Anxiety is a natural adaptive emotion that has allowed humans to survive in the face of threatening situations (Gutiérrez-García & Contreras, 2013). If, however, this emotion presents without a threat, and persists for a long period of time, it can become pathological. The term anxiety is broad, and anxiety disorders as an umbrella term combines many specific pathologies within it, including generalised anxiety disorder, panic disorder and specific phobias as diagnosed by the Diagnostic and Statistical Manual for mental disorders, version five (American Psychiatric Association, 2013).

Each of these disorders have their own characterisations. *Generalised anxiety disorder* reflects an excessive, uncontrollable worry on multiple issues on most days, spanning a period of six months or more. *Panic disorders* are classified by the occurrence of panic attacks that cause major worry for one month or more and significant behavioural changes. *Specific phobias* are characterised by fear, worry and avoidance in the presence or anticipation of a particular object or situation (e.g., spiders, heights). *Social anxiety disorder* involves fear of being embarrassed, humiliated, rejected or looked down upon in social interactions (e.g., public speaking, meeting new people) and subsequent avoidance of them

that last for more than six months. *agoraphobia* reflects the worry and fear of being in situations where escape may prove difficult or embarrassing (e.g., using public transport, being in a crowd). The fear experienced is often out of proportion to the actual situation, lasts generally six months or more and causes problems in functioning.

Separation anxiety disorder involves fear and worry about the separation from home or a major attachment figure. Although traditionally diagnosed in children, the DSM-5 has broadened the diagnostic criteria to allow for adult-appropriate symptoms. For individuals younger than eighteen, separation anxiety symptoms need to persist for at least 1 month, whereas for adults this required duration is at least 6 months. Finally*, selective mutism* involves the inability to speak in certain situations (e.g., at school, socialising with friends) despite being able to speak normally in other settings (e.g., at home). The symptoms must last more than a month and not be related to other speech difficulties. The two disorders described above previously belonged among disorders occurring in childhood but have been moved to the anxiety disorder category and adjusted to reflect their adulthood presentation. Obsessive compulsive disorder (OCD) and post-traumatic stress disorder (PTSD) have historically also belonged to the anxiety disorder category but now appear under their own category in the DSM (Kupfer, 2015).

Although clinically diagnosed disorders offer a validated approach, they have their disadvantages. Disputes in diagnostic boundaries and comorbidity between disorders are among the major drawbacks of this kind of categorisation (Widiger & Samuel, 2005). In addition, reaching out to clinical populations for research is not always feasible. Hence, research into psychiatry has adapted more and more to a symptom-based approach, offering a

dimensional view on the psychiatric spectrum. This dimensional conceptualisation can also allow more inclusivity, catering to those that may traditionally fall sub-threshold in diagnoses. This view has therefore gained substantial recognition overtime and is seen as more advantageous over a categorical model (Bjelland et al., 2009; Carragher et al., 2015; Insel et al., 2010; Widiger & Samuel, 2005). In this thesis, we adopt this dimensional perspective, focusing on anxiety symptoms as normally distributed in the general population.

1.2.1. Prevalence

Anxiety disorders are highly common. It is estimated that 14% of the European Union population are affected by anxiety disorders, which approximates to over 8 million people (Wittchen et al., 2011). In the UK, it is estimated that anxiety disorders affect up to 18% of the population (Fineberg et al., 2013). Globally, the lifetime prevalence of anxiety disorders range from 7-16% (Bandelow & Michaelis, 2015; Baxter et al., 2013; Remes et al., 2016). The prevalence of anxiety disorders are also higher in those with comorbid physical illnesses in comparison to healthy samples (Mitchell et al., 2013).

Symptoms of anxiety are also highly prevalent; it is estimated that in older adult community and clinical samples, 15-56% of individuals experience them (Bryant et al., 2008). Many of these symptoms are unrecognised and untreated, especially if they do not conform to or fall short of the traditional diagnostic criteria.

1.2.2. Age of onset & course

Anxiety disorders can manifest in childhood as early as 5 years, mostly through separation

anxiety disorder and specific phobias (Bandelow & Michaelis, 2015). Panic disorder and generalised anxiety disorder usually have a later onset in comparison to others (Kessler et al., 2007). A recent study finds that the earliest onset for anxiety/fear related disorders was 5.5 years, with a median onset of 17 years (Solmi et al., 2021). Although there is a general decline, anxiety disorders are also seen in older populations (Mehta et al., 2003). Anxiety disorders are therefore frequent and can manifest very early on in life.

The disorders are typically chronic, meaning that individuals live with the disorder for many years or decades. They do not, however, always last a lifetime. Treatment strategies, including pharmacological treatment e.g., anxiolytic medication and psychological therapy, e.g., cognitive behavioural therapy (CBT) can improve individuals' condition (Craske et al., 2017). It often takes many years for people to seek and access treatment for anxiety disorders (Baldwin et al., 2012).

1.2.2. Aetiology

Anxiety is classified as a complex trait, meaning that it is influenced by many different aetiological factors. For the purposes of this thesis, the aetiology of anxiety will be discussed considering two broad topics: genetics and the environment. There is, however, an abundance of other factors that can contribute to the development of anxiety including neural mechanisms, which although important, is not the primary focus in this thesis.

Twin studies have been an invaluable tool to investigate the genetic loading of anxiety disorders (see chapter 2 for a detailed explanation of the twin method). In essence, twins pose

a natural experiment, whereby identical twins share 100% of their DNA whereas non-identical twins share, on average, 50%. Hence comparison of these sets of twins on concordances can allow estimation of the genetic and environmental liability to a trait in a given population at a given time. This genetic liability is also known as heritability, i.e., the proportion of variance in a trait that can be attributable to genetic differences in the population. Heritability estimates of anxiety disorders range around 30-50% (Guffanti et al., 2016; Scaini et al., 2014; Shimada-Sugimoto et al., 2015). The risk of developing an anxiety disorder is also 4-6 times higher when a first degree relative is affected by the disorder (John M. Hettema et al., 2001). Heritability of anxiety and related internalising symptoms range between 30 – 70% with genetic effects found to contribute to the stability and continuity of these traits (Gillespie et al., 2004; López-Solà et al., 2014; Nivard et al., 2015; Trzaskowski et al., 2012).

As well as twin studies, there has been investigations of candidate genes, mostly in relation to anxiety disorders. Genetic variants such as the 5HTTLPR polymorphism of *SLC6A4*, as well as *COMT*, *BDNF*, *GABA-ergic* and *GAD* genes have been implicated in anxiety literature (Hartley & Casey, 2013; J. M. Hettema et al., 2015; Lacerda-Pinheiro et al., 2014; Smoller, 2016). Many studies have focused on polymorphisms of the serotonin transporter protein (5HTTLPR) coded for by gene *SLC6A4*, due to its influence in regulating the neurotransmitter serotonin in anxiety (Kenna et al., 2012). Namely, the short 'S' allele compared to the long 'L' allele may be associated with risk for developing anxiety and fear related phenotypes. It has also been argued that the influence of this genetic variant is more apparent when combined with environmental stressors, known as gene environment interplay, described later in this section. The candidate gene approach, however, has its limitations. One

of the most important drawbacks has been the failure to replicate many of the work,

compromising reliability of results. In addition, given that anxiety is a complex trait, it is not

sufficient to focus on a few genetic loci.

Instead of looking at specific genes, research has evolved to scan the whole genome for

common genetic variants, overcoming limitations of the candidate-gene approach (Duncan et

al., 2019). Genome-wide association studies (GWAS) test whether single nucleotide

polymorphisms (SNPs) can be associated with a trait on a global, genome-wide level.

Polymorphisms are genetic variations in a portion of DNA that occurs in a population at a

frequency usually greater than 1-5%, leading to multiple alleles or versions of a given gene.

In early GWAS, SNPs often did not reach genome-wide significance or were suggestive at

best (Erhardt et al., 2011; John M. Hettema et al., 2011; Otowa et al., 2012; Schosser et al.,

2013; Shimada-Sugimoto et al., 2015; Trzaskowski et al., 2013; Walter et al., 2013).

Detecting this type of common genetic variation requires substantial sample sizes for

sufficient statistical power.

With technological advances and collaborative efforts amongst scientific teams, recent

studies with larger samples have begun to show significant genetic associations with anxiety

disorders (Meier & Deckert, 2019; Otowa, Hek, et al., 2016; Purves et al., 2020; Stein et al.,

2017). Top 'hits' (as ranked by the significance of p-values) from these GWAS have been

identified including those located in genes *THBS2* and *CAMKMT* (Gottschalk & Domschke,

2017). Anxiety related phenotypes have also been researched using GWAS. Several meta-

analyses reveal significant top hits for neuroticism (de Moor et al., 2015; Okbay et al., 2016;

Smith et al., 2016), found to be highly correlated with anxiety both phenotypically and

genetically (Hansell et al., 2012; Okbay et al., 2016). This suggests the value of investigating the highly connected molecular network of the anxiety spectrum. Overall, the genetic influence on anxiety seems to be additive, with common variants each with small effect sizes aggregating to increase the risk of developing an anxiety disorder.

A natural next step, polygenic risk scores (PRS) can be calculated based on GWAS results to inform on individual liability to an anxious disorder. As opposed to previous methods to estimate heritability, i.e., genetic influences on variance (individual differences) in the population, PRS represent personalised risk scores. The method involves combining all SNPs associated with a disorder, weighted by their genome-wide significance, to form an individual 'score' based on their genotype. Large sample sizes and consistent replications are required to confirm the loci found before confidently applying these scores. Studies have begun to show that anxiety could be predicted from polygenic risk scores of other disorders such as psychosis (Sengupta, 2017). Researching the underlying genetic liability towards an anxious disposition can ultimately inform research and clinical practice.

Studies also suggest considerable genetic overlap between the different anxiety sub-types (Loken et al., 2014; Maron et al., 2010; Roberson-Nay et al., 2012; Smoller et al., 2008), indicating shared genetic aetiology across anxiety disorders. Significant genetic overlaps have also been reported with other psychiatric traits such as depression and neuroticism (Morneau-Vaillancourt et al., 2020; Ohi et al., 2020). This perhaps reflects the 'generalist genes hypothesis', whereby the similarity between anxiety disorders and other phenotypes are driven by widespread genetic effects (Brown et al., 2014; Eley, 1997; Kovas & Plomin, 2006). Investigating these genetic influences can not only provide insights into anxiety but

also inform on the various co-morbid traits.

The evidence both from twin and molecular genetic studies highlight significant genetic aggregation of anxiety. The role of the environment, however, is also a major contributing factor that accounts for variability in anxiety.

1.2.3. Environmental factors in anxiety

As well as genetic effects, environmental factors have been a research focus in relation to anxiety disorders and trait anxiety. This is especially given that heritabilities for anxiety traits and disorders never reach 100% and notable discordance observed within genetically identical twin pairs. The importance of environmental effects on symptoms of anxiety is found to increase with age and found to be less stable than genetic effects (Nivard et al., 2015). Various different environmental factors have been associated with risk of developing anxiety and can show innovations at each age, shaping lifetime risk. It is, however, often difficult to disentangle environmental effects from genetic ones, as many environmental factors are also partly influenced by genetic factors. In addition, measuring the environment also poses a challenge. Self-reports of environmental influences are cheap and practical but poses problems in terms of accuracy and recall bias (Poulton et al., 2008). Hence, it is important to note that the factors listed below are *a)* not exhaustive *b)* may not be 'purely' environmental and should also be interpreted in the context of genetic confounding and *c)* may not have been measured/recalled objectively and accurately.

Pre-natal environment

The pre-natal environment has been linked with various health outcomes. Maternal

psychological distress including daily stressors, pregnancy-induced anxiety, severe life events

(e.g., bereavement) or being affected by natural disasters and war have been associated with

adverse stress response systems (Glover et al., 2010). Prenatal stress may reprogram the HPA

axis, leading to increased secretion of stress hormones in the offspring (Glover et al., 2010;

O'Connor et al., 2005). Maternal stress may also be associated with anxious phenotypes in

the offspring (Betts et al., 2015; Glover, 2014; Schmitt et al., 2014). However, as mentioned

above, it is difficult to establish causality with these studies. Potentially confounded by

genetic transmission (Glover, 2014), we may not be gauging a 'pure' environmental factor.

Adverse life experiences

Negative life events have been a major environmental factor studied in relation to anxiety

pathology. Traumatic experiences have been reported in a high proportion of those with an

anxiety disorder (Norton & Abbott, 2017), and is also found to influence comorbid affective

and psychotic symptoms (van Nierop et al., 2015). Victimisation and maltreatment, including

physical and sexual abuse, are amongst major traumas contributing to elevated levels of

anxiety disorders (Norton & Abbott, 2017; Rapee, 2012, 2015). Bullying victimisation is

associated with higher odds of social anxiety and separation anxiety disorders (Silberg et al.,

2016). In fact, there is strong evidence to suggest causal relationships between bullying and a

wide range of mental health problems including anxiety, as measured dimensionally and

clinically (S. E. Moore et al., 2017; Silberg & Kendler, 2017; Singham et al., 2017). On the

contrary, a supportive family and social network can be a protective element for anxiety

symptoms, acting as a potential buffer (Festa & Ginsburg, 2011; Howell & Miller-Graff,

2014; Tyler et al., 2018). This again reiterates the influence of environmental factors in

anxiety disorders and highlights the role of positive parenting.

At a cognitive level, victimisation has been argued to influence anxiety symptoms (particularly social anxiety) through social learning processes, emotional dysregulation and maladaptive cognitive strategies, such as self-blame and rumination (Garnefski & Kraaij, 2014; Poole et al., 2017; Swearer & Hymel, 2015). At a biological level, trauma is argued to alter hormonal signalling and the limbic system, contributing to anxiety symptoms (Rivara et al., 2016). In addition, adversity during sensitive periods are argued to exacerbate anxiety phenotypes due to potential changes in the amygdala (Pechtel et al., 2014).

Parenting

Parenting has been an area of debate considering anxiety, and an area with the most potential genetic confounding. Studies have suggested that a negative parental style is linked to clinically relevant anxious symptomatology (Meyer & Kroner Herwig, 2017). One of the most studied dimension in terms of parenting, overcontrolling, authoritarian and critical parenting have been consistent predictors of social anxiety disorder (Gulley et al., 2014; Norton & Abbott, 2017; Scaini et al., 2018). Parental coldness and protectiveness has also yielded significant associations with generalised anxiety disorder and phobias (Otowa et al., 2013). Parenting therefore seems to play a crucial role on offspring anxiety, but what are the mechanisms? It has been argued that overcontrol can mean restrictions in exploring the environment, making the child vulnerable in fearful situations (Brook & Schmidt, 2008; Scaini et al., 2018). Formation of an insecure attachment can also put individuals at risk of developing an anxiety disorder and other psychiatric problems later on (Colonnesi et al., 2011; A. Lee & Hankin, 2009).

Parenting can also be a protective factor in the face of anxiety. For instance, supportive parenting has negative associations with anxiety disorders and parental warmth has been linked with resilience to aversive experiences (Lind et al., 2018). This implies parenting as a potential moderator of the effects of adversity, being a possible area for intervention strategies. However, it is unclear whether parenting behaviours are causal factors, a response to their child's existing symptoms, or most likely: a result of a complex gene-environment interaction (Gouze et al., 2017; Q. J. J. Wong & Rapee, 2015). For instance, genetically driven anxious symptoms may interact/evoke parental behaviours that further amplify the disorder (see section on gene-environment interplay).

Socioeconomic status (Income, Education, Neighbourhood/area)

Family socioeconomic status (SES) has also been an area of interest, although again most likely to be confounded by genetic effects. Individuals with a low-income background are found to have a higher prevalence of anxiety disorders (Rapee, 2012). Poverty-related stress is found to be a significant contributor towards anxiety symptoms (DeCarlo Santiago et al., 2011). Socioeconomic factors such as level of education and living conditions also show associations with anxiety, specifically generalised anxiety disorder (Ansseau et al., 2008). Other potential risk factors for anxiety disorders include being divorced, separated, or widowed as well as unemployment (Cheung & Yip, 2015; J. O. Lee et al., 2019). Living in a positive neighbourhood during childhood is found to reduce the risk of developing an anxiety disorder 20 years later (J. O. Lee et al., 2019). On the contrary, neighbourhood disadvantage (e.g., low social cohesion and safety, more traffic and social beneficiary claiming) has a link with anxiety disorders (Generaal et al., 2019). Hence low socioeconomic status can be a

central environmental influence on anxious symptoms and anxiety disorders. However, studies have also suggested large genetic influences on SES and several interactions that may mean that individuals may be more vulnerable to the effects of low SES (Sadeh et al, 2010). This therefore highlights the potential role of gene-environment interplay in understanding anxiety in its symptomatic and clinical form.

1.2.4. Gene environment interplay in anxiety

Gene environment interaction

According to early diathesis – stress models, risk of anxiety and other psychiatric traits can increase as a result of a genetic predisposition, exacerbated by the environment. Also known as a gene-environment interaction, GXE, there has been studies indicating that a genetic vulnerability can interact with environmental factors (e.g., stress) to influence anxiety. One genetic variant which has been extensively studied is the polymorphisms of *5HTTLPR* located in gene *SLC6A4*. Studies have shown that those with two short alleles (SS) may be more vulnerable to the effects of adversity (compared to short/long; SL and two long; LL alleles). Adversities such as stress, bullying and maltreatment and even experimentally induced psychosocial stress have been found to moderate the influence of *5HTTLPR* on anxious mood and pathology (Gunthert et al., 2007; Sun et al., 2020). It has therefore been argued that certain genetic effects may be more pronounced alongside environmental factors.

This gene-environment interaction has also been reported for related traits such as neuroticism and emotional problems (Pluess et al., 2010; Sen et al., 2004; Sugden et al., 2010). However, findings on GxE in anxiety remain relatively sparse and also report

conflicting findings (Kenna et al., 2012; Laucht et al., 2009). For instance, in a sample of

Chinese adolescents, the L allele (as opposed to the S allele) of *5HTTLPR* has been shown to

confer risk for anxiety symptoms in the face of stressful life events (Ming et al., 2015). This

therefore suggests the lack of consistent findings and also that results may not be

generalisable to other populations and ancestries.

For other genes, significant interactions have been found between variants of the Oxytocin

receptor gene (*OXTR*) and maltreatment. G-allele carriers (compared to the A allele) are

found to be more vulnerable to anxiety only when combined with this kind of environmental

adversity (Hostinar et al., 2014; Onodera et al., 2015). Individuals with the GG allele are also

reported to have altered limbic structures and increased responsiveness to emotion

(Dannlowski et al., 2016; Tost et al., 2010). The *OXTR* genotype may therefore influence the

receptiveness (structurally and functionally) to social environmental stimuli. Experiencing

trauma in the form of a natural disaster is found to interact with genotype. For instance, an

increased risk for GAD was reported in those affected by a hurricane and a single-nucleotide

polymorphism (SNP) of the *NPY* gene (Amstadter et al., 2010). Again, research has

warranted caution when interpreting GxE findings in relation to the *OXTR* gene (Kogan et al,

2011).

Interactions between the short variants of the *MAOA* gene and childhood maltreatment was

associated with increased anxious apprehension (Baumann et al., 2013). Furthermore,

polymorphisms of the *CRHR1* gene shows interactions with maltreatment to increase

neuroticism, a closely related trait to anxiety (DeYoung et al., 2011). Homozygosity for the

Met allele in *COMT* and T allele in *NSPR1* have also been found to confer risk for anxiety

sensitivity when coupled with childhood trauma (Baumann et al., 2013; Klauke et al., 2014). Considering the latter however, findings also suggest that females with the A allele (as opposed to the T) of the *NSPR1* gene were more frequently experiencing affective/ anxiety disorders when also exposed to stressful life events (Laas et al., 2014), suggesting diverging results. This also points to the role of sex in the context of anxiety GXE, alongside factors such as culture and ethnicity (Kim et al., 2011). Overall, candidate gene-environment interaction provides an alternative approach to delve into the complexity of anxiety, although there are several methodological and theoretical issues to consider when interpreting findings. Below, I summarise some of the key limitations of the candidate gene design.

Limitations of the candidate gene approach

Firstly, anxiety as with all complex traits, is unlikely to be explained by single-gene paradigms, and we can only conclude that genetic variations contribute to the overall *risk* alongside other genetic and environmental factors (Nugent et al., 2011). We now know that complex psychiatric traits are highly polygenic and require thousands of genetic variants with small effect sizes aggregating to increase this risk. Second, the inability to replicate previous findings is particularly concerning. A systematic genome-wide GxE analysis for depression reported no significant genome-wide hits and no support for previously known candidate genes (Van der Auwera et al., 2018). This is also reiterated in other systematic studies suggesting that early hypotheses about candidate genes were incorrect and that the large number of associations reported in the literature are likely to be type-1 errors, i.e., false positives (Border et al., 2019; Dick et al., 2015). Several reasons may exist for low replicability, including small scale studies with low statistical power, publication bias (in favour of novel candidate GxE studies) and low probability of the given hypothesis being true

(Duncan & Keller, 2011). This leads onto a third limitation; the approach requires a strong biological basis for selecting an appropriate gene/ polymorphism. There is, however, limited understanding of exact biological mechanisms underlying anxiety and other psychiatric disorders (Assary et al., 2018). The risk of selecting inappropriate candidate genes is therefore high, especially given that previous results may seem more robust than they are and are likely to be false-positives (Bosker et al., 2011).

Finally, majority of candidate GxE studies have taken a diathesis-stress focus whereby a genetic vulnerability to a particular trait in combination with a negative environment exacerbates risk of developing a psychiatric trait. Yet, studies also suggest an alternative (vantage sensitivity hypothesis), such that a genetic vulnerability may mean that some individuals are more likely to benefit from positive environments, in turn reducing risk of psychological distress and improved functioning (Pluess, 2017). Most studies have focused on the former approach, potentially providing a biased viewpoint on GxE. Given these limitations, it is no surprise that the field of behavioural and psychiatric genomics have transitioned to polygenic and genome-wide approaches to test GxE.

Gene-environment correlation

As opposed to sensitivity to the environment based on genotype, a gene environment correlation (rGE) involves exposure to the environment based on genotype (Plomin et al., 1977). There are three main types of gene-environment correlation. *Passive* gene environment correlation refers to the exposure a child can have based on genetic tendencies of their parents. For example, parents' genetic tendencies towards anxiety means that the offspring will not only inherit a propensity for internalizing problems, but also experience an

environment that enhances their likelihood of developing such problems such as through overcontrol. *Evocative* gene environment correlation refers to the child's behaviour (influenced by genotype) evoking a response from the environment. For instance, if a child has a genetic disposition towards anxiety, they may display irritability which elicits a specific parental response such as emotional overinvolvement. Finally, an *active* rGE refers to the individual seeking environments that correlate with their genetic tendencies. For example, someone with anxiety may avoid environments fostering socialising, correlating with their genetically driven socially anxious behaviour.

Although sparse, some studies have identified gene-environment correlations in relation to anxiety phenotypes. Research implies the role of the dopamine receptor gene (*DRD2*) with a significant rGE reported between the Taq1A polymorphism of the *DRD2* gene and parenting behaviour. Children with the A1 allele in this polymorphism were found to receive less supportive parenting and displayed more negative emotionality during a series of laboratory tasks (Hayden et al., 2010). It has been suggested that this may be a result of evocative rGE (parents responding to child's negative affect) or passive rGE (parenting style a result of parents' genotype, also passed to child).

Maternal overcontrol as part of an evocative rGE has been investigated further. For instance, children who experienced extreme maternal control in a laboratory task were more likely to report higher anxiety levels (Eley et al., 2010). Aside from shared genetic risk factors, it has been argued that this correlation may arise due to high child anxiety eliciting parental control. Parental overcontrol has been associated with persistent anxiety through to adolescence (Borelli et al., 2015). Genetically driven anxious symptoms can therefore elicit a specific (in

this case, controlling) parenting style that can persist through developmental trajectories. Research also suggests that mothers were less involved and were less negative when interacting with children classified as non-clinical compared to children with anxiety (Hudson et al., 2009) (Hudson, Doyle & Gar, 2009). Maternal overinvolvement and negativity may therefore arise as a result of higher anxious behaviour. This could again reflect an evocative rGE whereby the mother shows differential treatment to the child based on the child's anxious behaviours.

The twin design, whereby perceived parenting is modelled as a characteristic of the individual, similar to IQ or personality, makes it possible to explore the extent to which it is affected by genetic (rGE) and nongenetic influences (Plomin & Bergeman, 1991). A meta-analysis of studies of this type reports a heritability estimate of 23% (Avinun & Knafo, 2014), implying that genetic influences that stem from the child can shape parental behaviour. The children-of-twins design has also been used to understand the role of parenting in children's internalising behaviour. A systematic review and meta-analysis suggests that the association between maternal anxiety and offspring internalising problems was mainly as a result of genetic transmission rather than through non-genetic effects (Ahmadzadeh et al., 2021), mirroring previous work (Jami et al., 2020). This points to the role of genetic transmission in influencing anxiety, pointing to potential passive rGE in parenting behaviours.

As well as parenting behaviours, research indicates that a genetic predisposition to anxiety may evoke a response from peers. For instance, findings suggest that children with genetically driven anxiety were more likely to be victimised by other peers (Brendgen et al.,

2015). Deemed as an evocative rGE, it is argued that children with inherited anxious behaviours may be viewed as more submissive and less able to defend themselves. However, as is the case with all correlational research, we cannot infer causal mechanisms. Also, the same genetic factors that link anxiety with victimisation can also influence other traits that influence both, being a mediator of the relationship.

The presence of gene-environment correlations can also be seen in terms of friendship groups. Findings suggest that individuals with a strong genetic tendency towards anxiety were more likely to have anxious than non-anxious friends (Poirier et al., 2016). This points to a potential active rGE whereby individuals seek friendships that resemble their own behavioural characteristics (Guimond et al., 2014; Rubin et al., 2006). This could also reflect an evocative rGE, whereby anxious individuals may evoke a negative response from non-anxious peers. Genotype-environment correlations can therefore be useful although disentangling and measuring the influence of each type can be difficult.

1.3. Depression

Depression in its clinical form is characterised by cardinal symptoms of low mood and loss of interest or pleasure (anhedonia). Major Depressive disorder (MDD) requires the presence of five or more symptoms within a 2-week period including one of the cardinal symptoms with secondary symptoms of appetite or weight changes, sleep difficulties (insomnia or hypersomnia), psychomotor agitation or retardation, fatigue or loss of energy, reduced ability to think or concentrate, feelings of worthlessness or excessive guilt, and suicidality (American Psychiatric Association, 2013).

This approach, however, ultimately informs on a binary diagnosis, with a depressive episode present or not. As with anxiety, however, depression is increasingly viewed as a dimensional, complex trait with various sub-types (Tolentino & Schmidt, 2018). Many studies therefore often use the symptoms mentioned above to measure depression severity in individuals, capturing its presence as a normally distributed trait in the population. In this thesis, we adopt this symptom-based approach to measuring depression in chapters 5 and 6. A full review of depression is out of scope for this thesis. Here, I provide a brief overview of what is known about the epidemiology and aetiology of depression.

1.3.1. Prevalence

Depression is a highly common mental health condition with a lifetime prevalence of around 10% in the general population and estimated to reach up to 20% in clinical populations (Kessing, 2007; Kessler & Bromet, 2013; Richards, 2011). It is also a global problem, affecting individuals from low to high-income countries, though prevalence rates are often higher in the latter group (Lim et al., 2018). As well as income, depression is often found to be higher in women compared to men (Van de Velde et al., 2010).

Depression frequently co-occurs with other mental health conditions, with the most common being anxiety disorders (Lamers et al., 2011). This comorbidity is related to greater severity of symptoms, lower treatment response and quality of life, as well as potentially obscuring the true prevalence of depression and/or anxiety in the population (Johansson et al., 2013). A review of this comorbidity can be found in section 1.4.

1.3.2. Aetiology

As a complex trait, there are various factors that contribute to the cumulative risk of developing depressive disorders. Here, I provide an overview of the genetic and environmental contributions. Twin studies estimate that major depression is moderately heritable with an early meta-analysis indicating an aggregate estimate of 37% (Sullivan et al., 2000). A later meta-analysis indicates that heritability of a depressive episode to be around 40% (Polderman et al., 2015). As with anxiety, candidate gene studies have not demonstrated a substantial contribution of specific genes to depressive disorders and have often failed to replicate (Border et al., 2019; Bosker et al., 2011; Luo et al., 2016). It is argued that phenotypic and genetic heterogeneity of depression could contribute to this. On the other hand, GWAS studies have begun to highlight the role of many individual common genetic variants to the liability of depression both in clinical and symptom form (Howard et al., 2019; Levey et al., 2021; Mullins & Lewis, 2017; Wray et al., 2018).

The aetiology of depression is further elucidated by longitudinal designs. An early study on women suggested that over a 1-year period, genetic effects on the liability to major depression were entirely stable, while environmental effects were transient and not likely to influence temporal stability of depression long term (Kendler et al., 1993). Later studies also indicate genetic innovation for anxiety and depression in early adulthood which is transmitted and explains genetic variation in mid to late adulthood (Gillespie et al., 2004; Nes et al., 2007). A more recent large longitudinal twin study suggests high heritability of depression and anxiety symptoms in childhood (60-70%), which decreases to around 40-50% in

adulthood. This decrease was explained by an increase in unique environmental variance rather than a decrease in genetic variance. Phenotypic stability of these symptoms was mainly attributed to genetic factors (Nivard et al., 2015). Genetic components of anxiety and depression therefore appear to be relatively stable across the lifespan.

1.3.3. Environmental factors in depression

There are various environmental factors that have been associated with risk of developing clinical depression. These include and are not limited to parenting, neighbourhood factors, traumatic events, and socioeconomic status (SES). It is noteworthy however, that as mentioned previously, factors such as parenting, and SES can also be confounded by genetic influence and therefore gauging pure environmental factors in relation to depression is difficult. One way to get closer to pure environmental influence is through adoption studies and children-of-twins designs which can estimate the influence of a trait over and above genetics. A review study of these study designs finds that parenting, more specifically maternal depression, is a risk factor for the emotional, behavioural, and neurobiological development of children (Natsuaki et al., 2014). Another method to gauge pure environmental influence is mendelian randomization, which is an analogy to randomised control trials and largely overcomes the problems of genetic confounding and reverse causality (Gage et al., 2013). One such study identifies six environmental risk factors (widowhood, childhood physical abuse, obesity, having 4–5 metabolic risk factors, sexual dysfunction and job strain) that can influence clinical depression through this method (Köhler et al., 2018). Below, I summarise some of the main environmental factors that have been consistently linked with risk of developing depressive disorders.

Adverse life experiences

As with anxiety disorders, adverse, traumatic, and stressful life experiences can contribute to risk of developing depression. Studies both in community-based samples and clinical populations indicate that those with a history of stressful life events and adversity, especially early on in life, were more likely to develop depression in adulthood (Maughan et al., 2013; McLaughlin et al., 2010; Saveanu & Nemeroff, 2012; Thapar et al., 2012). Childhood trauma has also been linked with severity of psychological distress, such that childhood trauma can be three times more prevalent in individuals with comorbid anxiety and depression than controls (Hovens et al., 2010).

Traumatic life experiences have also been shown to impact the clinical course of depression. For instance, those with depression and a history of childhood trauma are reported to have earlier onset of depressive symptoms, longer depressive episodes, a more chronic illness course and lower rates of remission and recovery (Hovens et al., 2012). Overall, stressful and traumatic events, particularly in childhood and adolescence, negatively impacts individuals psychological wellbeing and significantly increases the risk of depression onset.

Parenting

Parenting and the home environment can also be an important factor in the development and course of depression. For instance, a meta-analysis of risk factors for depression suggests that emotional abuse and neglect were the strongest predictors of adult depression over other kinds of childhood trauma (Mandelli et al., 2015). A recent systematic review indicates other significant influences such as parental overcontrol, hostility, harsh parenting and low positive

affect (Clayborne et al., 2021). In another study, higher levels of parental conflict and aversiveness were associated with increased risk for both depression and internalising problems. Higher levels of abusive parenting, over-involvement and lower levels of warmth were also found to be risk factors for internalising symptoms (Yap & Jorm, 2015).

Parental depression is also a potential factor in increasing offspring depression risk. Aside from direct inheritance of genes, children of parents with high liability to depression can also be passively influenced by family environment factors such as negative parenting behaviours (e.g., authoritarian), interparent/marital conflict and sleep-diet patterns (Galbally & Lewis, 2017). The potentially modifiable parental factors mentioned above could be a valuable resource for intervention targets to reduce risk of anxiety and depression as done by others (Muzik et al., 2015; Yap et al., 2019).

Socioeconomic status (SES)

Factors such as income, level of education and neighbourhood factors have all been linked with depression risk. For instance, living in a deprived area, low level of education, experiencing financial strain and low household income have all been associated with higher risk of depressive symptoms and episodes (Ibrahim et al., 2013; J. L. Wang et al., 2010). This relationship between low SES and high depression has been observed in different age groups (Letourneau et al., 2013; Reiss, 2013) and across different populations (Domènech-Abella et al., 2018; Freeman et al., 2016). In general, a high income and education (both at individual and parental education levels) are seen as protective factors for depressive symptoms and disorders in the population (Bauldry, 2015; Goyal et al., 2010; Kosidou et al., 2011).

Most research in this area, however, has focused on western populations. There may be additional SES factors that can contribute to depression risk in other parts of the world. For instance, in a Chinese sample, education was associated with decreased depression risk, mirroring findings from the west, but additional community factors were also found to be highly relevant such as health infrastructure and social stress (Strauss et al., 2010). It is therefore important to consider environmental factors for depression under different cultural contexts.

1.3.4. Gene environment interplay in depression

Gene-environment interaction (GxE)

As with anxiety research, early work on GxE for depression focused on candidate genes and their interaction with environmental factors (e.g., stressful life events). The initial springboard for these studies has been from Caspi and colleagues suggesting an interaction between the 5HTTLPR genotype and stressful life events on depression (Caspi et al., 2003). Findings since then have been inconclusive. Meta-analytic studies focusing on the 5HTTLPR polymorphism found no interaction between genotype and stressful life events on depression (Munafò et al., 2009; Risch et al., 2009). A subsequent meta-analysis suggests strong evidence for a moderation effect (Karg et al., 2011) and a later analysis indicates a small but significant effect of 5-HTTLPR in interacting with stress for depression (Bleys et al., 2018). A collaborative meta-analysis of 31 datasets indicated no evidence for a strong interaction effect for depression and suggests that even if an interaction exists, it is not largely generalisable, must be of modest effect size and only observable in limited situations (Culverhouse et al., 2018).

Early twin studies also focused on interactions between candidate genes such as 5HTTLPR and environmental factors e.g., low SES and adverse life events on elevated depression risk (McAdams et al., 2013; Uher, 2014). Yet, the candidate gene approach has its limitations (as described previously), mainly due to low replicability and inconsistent findings. These inconsistencies have also been explained by issues in assessing environmental factors e.g., different types and measures of early life stress, as well as moderation differences observed across samples (Nugent et al., 2011).

As opposed to candidate gene studies, quantitative gene environment interaction twin models have explored this interaction further, in larger, better powered studies. One study finds significant moderation effects between different environmental risk factors and internalising symptoms, such that in the context of greater environmental adversity, nonshared environmental factors became more important in the aetiology of these symptoms (Hicks et al., 2009). Another study finds a significant GxE model whereby genetic influences on depression risk increased with increasing neighbourhood deprivation (Strachan et al., 2017). Similarly, a study finds that genetic influences on depression are moderated by sleep duration, such that further divergence from the normal range meant that genetic influences became more influential (Watson et al., 2014).

Research has also begun looking at whole genome approaches to GxE. Genome-wide environment interaction studies (GWEIS) search the entire whole genome for variants (SNPs) that could moderate the effects of the environment on psychiatric disorders. This hypothesis-free approach means that assumptions do not have to be made about a particular gene in

relation to depression. One such study explored interactions between SNPs with social support and stressful life events on depressive symptoms (Dunn et al., 2016). A genome-wide significant interaction was found between the SNP rs4652467 and stressful life events although the result did not replicate in a smaller independent sample. Similarly, another study reports an interaction between the SNP rs10510057 and stressful life events on depression risk, though this finding did not pass the more stringent threshold necessary for testing both main effects and interactions (Otowa, Kawamura, et al., 2016).

It is clear that GxE studies on depression have come a long way, and the field is adapting to more comprehensive analysis of the genome and environments. Small sample sizes and limited measures of the environment has meant few replicated GxE findings. A further step from GWEIS studies are the use of polygenic scores for depression and their interactions with environmental variables which have started to provide promising findings, especially when conducted longitudinally (Domingue et al., 2017; Mullins et al., 2016; Peyrot et al., 2014).

Gene-environment correlation (rGE)

As well as gene-environment interaction, there is also possibility of gene environment correlation for depression. For instance, one study finds bidirectional associations between child anxiety/depression & parental depression which could be potential evidence for an evocative rGE, whereby offspring internalising behaviours elicit an internalising response from the parents (Johnco et al., 2021).

In quantitative behaviour genetics, rGE is typically measured by gauging genetic influence on putative environmental factors. For instance, studies suggest that both depression and

stressful life events (especially those that are influenced by one's behaviour; i.e. dependent life events) are partly heritable (Boardman et al., 2011; Kendler & Baker, 2007). This may be evidence for active gene-environment correlation, whereby individuals with a genetic propensity towards depression may select into more stressful social situations. A later study provides further evidence for rGE such that family environment factors, namely family chaos and parenting style, were significantly heritable and significant genetic correlations were detected between depressive symptoms and these putative environments (Wilkinson et al., 2013).

1.4. Comorbidity between anxiety and depression

Given that most of this thesis discusses psychological distress under the umbrella of internalising symptoms, it is necessary to discuss the extant comorbidity between anxiety and depression. Research suggests overlap between the two traits both at phenotypic and genetic levels. Community and clinical samples both point to high prevalence of comorbidity, ranging from 15-75% (Cummings et al., 2014; Essau, 2008). This high co-occurrence is also noted regardless of age, with both younger and older populations affected by anxiety and depression (Schoevers et al., 2005; Wolitzky-Taylor et al., 2010).

Twin studies suggests that the anxiety-depression comorbidity is largely accounted for by shared genetic factors (John M. Hettema, 2008; Kendler et al., 2007; Middeldorp et al., 2005), with some suggesting that the two traits are almost genetically identical (Cerdá et al., 2010). More recently, genome-wide approaches have supported the high genetic correlations observed in twin and family studies. A recent genomic study finds a positive and significant

correlation between depression and anxiety and stress related disorders, identifying five pleiotropic loci simultaneously associated with the two traits (Mei et al., 2022).

Together, anxiety and depression can increase impairment and have negative implications for prognosis. One study reports high levels of anxiety-depression comorbidity and this was associated with more reported childhood trauma, higher neuroticism, longer duration of episodes, earlier age of onset and more severe symptoms (Lamers et al., 2011). Anxiety and depression, when combined, are therefore more severe, have a greater risk of suicide, more disabling, resistant to treatment, and result in more psychological, physical, social and workplace impairment than either disorder alone (Tiller, 2013). Their co-occurrence also increases risk of other psychiatric disorders, one of the most notable being substance use disorders (Lai et al., 2015).

As well as prognosis, the co-occurrence of anxiety and depression poses additional challenges for diagnoses. For instance, the clinical presentation of comorbid anxiety-depression can be more complex than 'pure' disorders, and individuals' anxiety and depression symptoms can fluctuate overtime (Wu & Fang, 2014). The traditional binary-style diagnostic categories are unable to capture this complexity, pointing once again to the value of a continuous approach, which allows all diagnoses to co-exist on a continuum.

1.5. Somatic symptoms

Somatic symptoms can comprise of various psychophysiological experiences, including headaches, muscle tension and heart palpitations. These symptoms can arise as a result of a

primary mental or physical health problems such as comorbid chronic pain as well as anxiety or depression. Researchers have used somatic symptoms as indicators of both psychological and physical health.

Twin studies indicate that somatic symptoms are heritable, with estimates ranging 11 – 54% (Gillespie et al., 2000; Hansell et al., 2012; Kato et al., 2009). Somatic symptoms are found to correlate with anxiety, neuroticism and depression both at a phenotypic and genetic level (Ball et al., 2011; Hansell et al., 2012; Vassend et al., 2012). In fact, it has been suggested that somatic complaints can be modelled alongside anxiety and depression under a common internalising factor (Ask et al., 2016; Simms et al., 2012). Multiple genetic variants (SNPs) have also been associated with somatic symptoms, independent of anxiety, depression or pain (Holliday et al., 2010).

Environmental factors such as traumatic life events and stress have also been associated with increased somatisation and somatoform disorders (Bonvanie et al., 2017; Creed et al., 2012; Crofford, 2007; J. Li et al., 2016; Rehna et al., 2016). One mechanism in which these environmental events can affect somatisation is through triggering dysfunction in hormonal activity and autonomic regulation (Kozlowska et al., 2020). Parenting has also been researched as part of a children-of-twins study, which can tease apart environmental effects over and above genetic influences of the parent and child. Findings show that parent-driven environmental effects explained the association between parental criticism and adolescent somatic symptoms (Horwitz et al., 2015).

Overall, somatic symptoms are a complex group of symptoms which are closely related to

anxiety and depression. Research suggests the suitability of modelling somatic complaints under a common internalising/ psychological distress factor (discussed in detail in chapter 5).

1.6. Relationship between psychological – physical health

Psychological distress is rarely just a mental health problem. Even at a surface level, anxiety and depression feature physical symptoms including heart palpitations, dizziness, loss of appetite and insomnia. The comorbidity between mental and physical health is a known phenomenon, with the two often co-occurring in clinical settings (Firth et al., 2019). Indicators of psychological distress, namely anxiety and depression, co-occur with a variety of physical diseases ranging from cardiovascular to gastrointestinal problems. In this section, I provide examples of this comorbidity and potential mechanisms underlying it.

1.6.1. Comorbidity between psychological distress & physical ill-health

Anxiety and depression symptoms and diagnoses have been associated with several diseases, including cardiovascular health problems, gastrointestinal diseases, and arthritis (El-Gabalawy et al., 2011, 2014; Matcham et al., 2016). The occurrence of heart disease, cancer, asthma, and chronic pain have all been found to be elevated in those with anxiety and depressive disorders (Stanton et al., 2019). The risk of physical health problems including persistent cough, asthma, hypertension, and gastrointestinal problems are also reported to be greater in those with comorbid anxiety and depression (Kang et al., 2017).

One of the best studied physical health domains in relation to psychological distress has been cardiovascular problems, including myocardial infarction (heart attack), cardiovascular and

coronary heart disease and cardiovascular mortality. Several studies suggest relationships between depression and reduced cardiovascular health (Cohen et al., 2015). One in five patients with coronary heart disease and heart failure is found to have a comorbid depressive disorder (Rutledge et al., 2006). These cardiac patients with comorbid depressive symptoms are also more likely to have physical limitations and poor quality of life, even after accounting for objective measures of cardiac function (Ruo et al., 2003). Depression has also been associated with increased risk of stroke (Dong et al., 2012; Meza et al., 2020; A. Pan et al., 2011) and hypertension (Z. Li et al., 2015).

There is, however, relatively less known about the anxiety-cardiovascular health relationship (Cohen et al., 2015). Studies generally suggest negative relationships between anxiety and indices of cardiac health (Allgulander, 2016; Celano et al., 2016, 2018; Player & Peterson, 2011; Tully et al., 2016). Meta-analytic findings suggest that initially healthy individuals with high levels of anxiety were at increased risk for incident coronary heart disease and cardiac death (Roest et al., 2010). Importantly, this risk remained even after accounting for demographic variables, biological risk factors, and health behaviours. A later meta-analysis reports a 52% increase in cardiovascular disease, independent of traditional risk factors (e.g., smoking and BMI) and depression (Batelaan et al., 2016). Longitudinal evidence also supports the inverse relationship between anxiety and cardiovascular health. One such study of Swedish men spanning over three decades indicates associations between anxiety disorder diagnosis and risk of coronary heart disease and heart attack (Janszky et al., 2010).

Another cohort study of Finnish men and women suggests links between anxiety and coronary heart disease over a seven-year follow-up period (Nabi et al., 2010). This

relationship was, however, completely attenuated in men following adjustment for confounders including sociodemographic characteristics and depression. Importantly, there are also reports of no associations between anxiety and cardiac-related hospitalisation and mortality (Versteeg et al., 2013) and even a potential protective effect of anxiety (Parker et al., 2011). It is therefore imperative for research and clinical purposes to further explore this anxiety-cardiovascular health relationship, especially in non-western populations given that most work centres around western, European samples.

As well as physical health conditions, psychological distress is known to influence health-related quality of life (HRQOL). Studies suggest significant negative associations between anxiety/depression and HRQOL, significantly impacting public health burden and cost of care (Beard et al., 2010; Gaynes et al., 2002; Saarni et al., 2007; Strine et al., 2005, 2009). In particular, comorbid anxiety and depression is found to substantially reduce mental and physical functioning (Johansson et al., 2013). Although essential, researching the prevalence of the mental-physical health comorbidity is not sufficient. In order to better understand and ultimately inform clinical practice, it is essential to explore the mechanisms affecting their relationship as well as explore possible causal links.

1.6.2. Potential mechanisms underlying this comorbidity

There are several possible mechanisms underlying the comorbidity between mental and physical health domains. Here, I provide an overview of three possible areas: the autonomic nervous system, hypothalamic-pituitary-adrenocortical (HPA) axis and the role of genetics.

Autonomic nervous system (ANS)

The autonomic nervous system (ANS) has been proposed as a potential moderator of the mind-body relationship. There are two main pathways within the ANS: sympathetic and parasympathetic. The sympathetic division prepares the body to act, also known as the 'fight-or-flight' response. The symptoms include an increased heart rate, sweating, and pupil and airway dilation. Bodily processes that are less important, such as digestion and urination, are inhibited. In contrast, the parasympathetic nervous system is involved in establishing balance or homeostasis. Adopting a 'rest-and-digest' approach, this division decreases heart rate and blood pressure and stimulates the digestive system, increasing energy storage (Robertson & Biaggioni, 2012). The autonomic nervous system is automatic meaning that the processes occur without conscious effort. **Figure 1.1** illustrates the two branches of the ANS.

Figure 1.1 – The two divisions of the autonomic nervous system and their functions
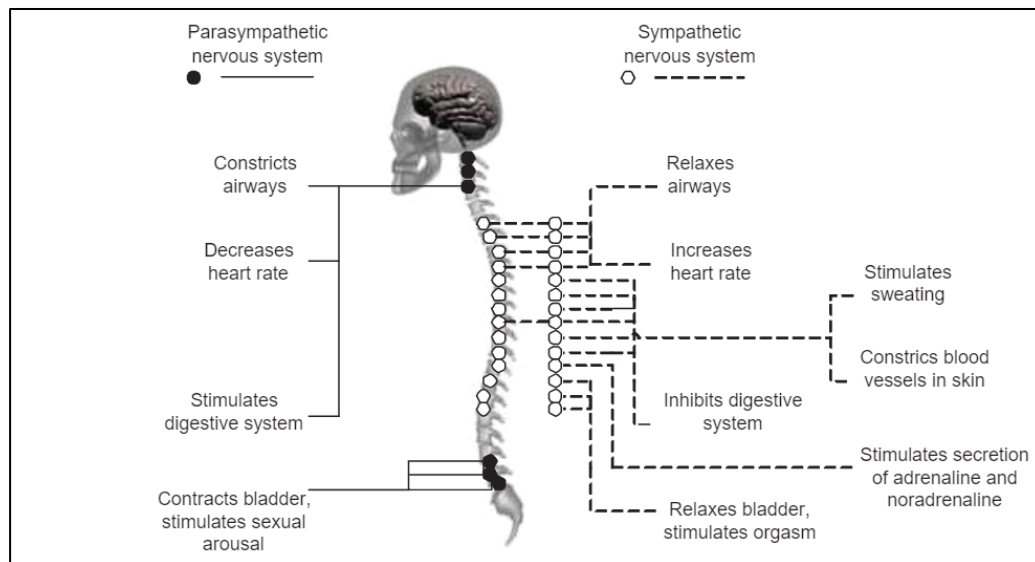
Image retrieved from Hempel, 2008

Research suggests that stress and stress-related disorders can contribute to dysfunctions of the

ANS, which in turn, can affect physical health (Gianaros & Jennings, 2018). Most of the work in this arena have focused on cardiovascular health. Psychological distress, including anxiety and depression is argued to induce exaggerated cardiovascular reactivity and an imbalance in the ANS divisions (Gianaros & Wager, 2015). Prolonged stress or chronic anxiety could potentially lead to an allostatic load putting individuals at risk of hypertension, heart attacks and heart disease (Carroll et al., 2012; Chida & Steptoe, 2010).

There are several other lines of evidence suggesting that autonomic dysfunction may be the bridge between anxiety and/or depression (both in its clinical and symptomatic forms) with cardiovascular health and mortality (Bajkó et al., 2012; Hu et al., 2016; Kop et al., 2010; Tolentino & Schmidt, 2018; Yiming Wang et al., 2013). Indices of cardiovascular health, such as heart rate variability shows substantial reductions considering psychiatric disorders and predominantly with anxiety (Alvares et al., 2016; Chalmers et al., 2014; Chang et al., 2013; Henje Blom et al., 2010; Pittig et al., 2013). One other study also suggests associations between cardiac autonomic dysregulation and anxiety/depression (Hu et al., 2019). This relationship, however, was found to be non-significant after controlling for antidepressant usage. This points to the role of potential confounders in the anxiety/depression – ANS relationship, meaning that more sophisticated designs are required to decipher this association.

HPA axis

It is suggested that the functioning of the hypothalamic-pituitary-adrenocortical (HPA) axis) can contribute to the risk of anxiety disorders and to the mind-body relationship. An integral

part of the stress response system, the HPA axis is involved in the release of hormones. In the face of an environmental stressor, the hypothalamus activates the release of corticotrophin-releasing hormone (CRH) from the hypothalamus. CRH stimulates the secretion of adrenocorticotropic hormone (ACTH) from the pituitary gland. Adrenal glands then produce glucocorticoids (cortisol in humans) in response to ACTH as well as activation of the sympathetic-adrenal system whereby adrenaline and noradrenaline are released (Micale & Drago, 2018). Cortisol is the primary stress response, often adaptive but can become damaging to organs if elevated chronically (Bao & Swaab, 2019).

Children with anxiety disorders have been found to have altered HPA axis functioning such as a higher skin conductance level and higher anticipatory and post-test arousal following experimental procedures (Dieleman et al., 2015; Faravelli et al., 2012). In line with this heightened hormonal activation, studies have shown increased HPA axis activity in depression and anxiety disorder subtypes (Juruena et al., 2020). One study suggested that abnormal HPA axis activity through higher cortisol secretion may be the mediator between depression and cardiovascular mortality in a sample of men (Jokinen & Nordström, 2009).

Another study, however, reported blunted cortisol reactivity in response to a cognitive stress task in those with early life stress and psychological distress history, while early life stress exposed individuals with little or no history of distress had significantly elevated baseline cortisol, prolonged responses, and greater total cortisol production (Goldman-Mellor et al., 2012). This suggests the differential hormonal reactivity based on psychiatric history and highlights the complex trajectories in which the HPA axis can interact with mental and physical health. In a later meta-analysis, however, early life stress was not associated with

cortisol (Fogelman & Canli, 2018), possibly due to high heterogeneity across studies. It has also been suggested that a potential moderator of this relationship is the type of stressor, such that some forms of trauma and distress may have a longer-lasting impact on the HPA axis (Fogelman & Canli, 2019; Menke et al., 2018). The HPA axis is therefore an important potential neurobiological mechanism that can underlie the mental-physical health association.

Genetics

As well as autonomic and hormonal regulation of the body, genetic factors can contribute to risk of poor mental and physical health. Most mental and physical health problems are complex, meaning that they can also be polygenic. Hence, the same genetic factors/ variants that predispose one to poor psychological health can also increase risk of developing a physical health condition. This polygenicity is evident through studies reporting several genetic correlations between symptoms of anxiety and depression with a variety of physical health problems including coronary artery disease, Crohn's disease and lung cancer (Levey et al., 2020; Lu et al., 2021; Purves et al., 2020). Genetic correlations have also been reported between major psychiatric disorders and body mass index (BMI) (Bahrami et al., 2020), and indicators of blood pressure and cholesterol (López-León et al., 2010), all major risk factors for cardiovascular health problems and obesity (Khan et al., 2018; Umer et al., 2017).

It is also possible that genetic differences in the population underlies the individual differences in cardiovascular reactivity and other psycho-physiological processes (Anokhin, 2014). For instance, one study reports genetic loci associated with heart rate variability, an indicator of cardiovascular health (Nolte et al., 2017). There are also genetic correlations

reported between anxiety disorders and several intermediary phenotypes such as brain region volumes that could be influential in the mind-body relationship (Ohi et al., 2020).

Although there is evidence for genetic underpinnings for both mental and physical health, research and especially behaviour genetic perspectives on the mental-physical health relationship remains sparse. Twin studies suggest genetic correlations between neuroticism and measures of cardiovascular health including heart rate variability (Riese et al., 2006, 2007). Yet, there is limited behaviour genetic literature combining mental and physical health domains, and especially limited work in non-western populations.

1.6.3. Cross-cultural differences in mental-physical health

Research suggests that mental and physical health can manifest differently in different cultures. Here, I'll highlight some of the key differences observed broadly across western and non-western populations. One of the main differences is cultural and societal attitudes to mental health problems. The stigma attached to mental health problems is a universal phenomenon, and still remains in western, more developed countries (Henderson et al., 2013; Thornicroft, 2006). Yet, an international study finds that the stigma experienced by those with mental health difficulties were significantly higher in developing rather than developed countries (Alonso et al., 2008). This suggests additional, culture-specific factors that are local and more prominent in non-western settings (Koschorke et al., 2017). Cultural stigma towards mental health can impact the way in which mental health is understood, contextualised, and ultimately treated.

Low mental health literacy is related to increased stigma and is another contributor to differences in mental health between western and non-western populations. Mental health literacy refers to the 'knowledge and beliefs about mental health problems which aid their recognition, management, and prevention' (Ganasen et al., 2008). Most studies in this arena, however, are focused on western samples such as in Australia and the UK, with 'large research gaps' found in non-western nations (Furnham & Hamid, 2014). Research efforts directed towards improving mental health literacy could potentially address disparities in mental health care in non-Western countries.

It is also found that those from non-western populations or from ethnic minority backgrounds may mask mental health difficulties as physical problems (Appel et al., 2011; Holden et al., 2014). For instance, regular heart palpitations mainly due to anxiety may be interpreted and treated for primarily as a cardiovascular problem. Disorders relating to psychological distress such as anxiety and depression may therefore be easily overshadowed by psychosomatic symptoms.

Culture-specific factors such as tradition, religiosity and spirituality are also possible influences on the way mental and physical health is viewed and treated (Koenig et al., 2012). For instance, research suggests that involvement of faith and community leaders in the awareness and treatment of mental and physical health problems can be advantageous (Koenig, 2012; Rodriguez et al., 2011; Weber & Pargament, 2014). Another cultural context to consider is the role of daily stressors, war, and conflict settings. Mental and physical health can show higher prevalence and treated very differently considering these ongoing and traumatic experiences (Dimitry, 2012; Miller & Rasmussen, 2010; Priebe et al., 2010). A

systematic review and meta analysis suggests high levels of depression prevalence in south Asian countries, and it is thought that this may be due to additional risk factors that may not be present/ as prominent in western populations such as urbanization, unemployment, substance abuse, natural disasters and political unrest (Bishwajit et al., 2017). It is therefore imperative that further research is taken to these non-western settings, to move towards more culturally informed adaptations of treatment (Napier et al., 2014).

1.7. Epigenetics

Up until now, the literature suggests that both genetic and environmental influences explain a substantial proportion of variance in indicators of psychological distress. These influences on their own however, do not explain 100% of the phenotypic variance, and even in identical twins there is evidence of discordance, suggesting the role of other factors. One way to explain this is through epigenetics. The term 'epigenetics' was first coined by Conrad Waddington in 1942, as the interaction between genes and their products controlling phenotypic changes that occur over the course of development (Waddington, 1942).

Epigenetics as broadly defined is the study of chemical modifications that happen above/on top of the DNA sequence, that can regulate gene expression without altering the original DNA code (Goldberg et al., 2007). It is worth noting that although epigenetics is often seen as a non-genetic mechanism, there is recent evidence to suggest that it can be heritable and epigenetic regulation can be underlined by genetic influence, e.g., in an allele-specific manner (Gertz et al., 2011; Kravitz & Gregg, 2019; M. I. Lind & Spagopoulou, 2018; Trerotola et al., 2015). Twin research is a valuable resource to uncover epigenetic heritability,

with both within and between twin pair analyses illustrating that epigenetic variation at specific genomic regions can be heritable and can show change over time (Bell & Spector, 2011; Kaminsky et al., 2009; C. C. Y. Wong et al., 2010).

There are various mechanisms in which epigenetic change can occur, including histone modifications and DNA methylation (Zhang & Pradhan, 2014). The latter is the most commonly researched epigenetic mechanism, and also forms the basis of chapter 6. I therefore provide an overview of DNA methylation and its relation to psychological distress here.

1.7.1. DNA Methylation

In mammals, DNA methylation involves the addition of methyl groups to cytosine bases in DNA. As this addition occurs at the 5th position of cytosines, this mechanism is often referred to as 5-methylcytosine. Majority of this DNA methylation occurs at cytosine bases that are followed by a guanine nucleotide, also referred to as a CpG site/ CpG dinucleotide (L. D. Moore et al., 2013). A visual representation of non-methylated versus methylated cytosine can be found at **Figure 1.2**.

Figure 1.2 - The chemical structure of cytosine and 5-methylcytosine
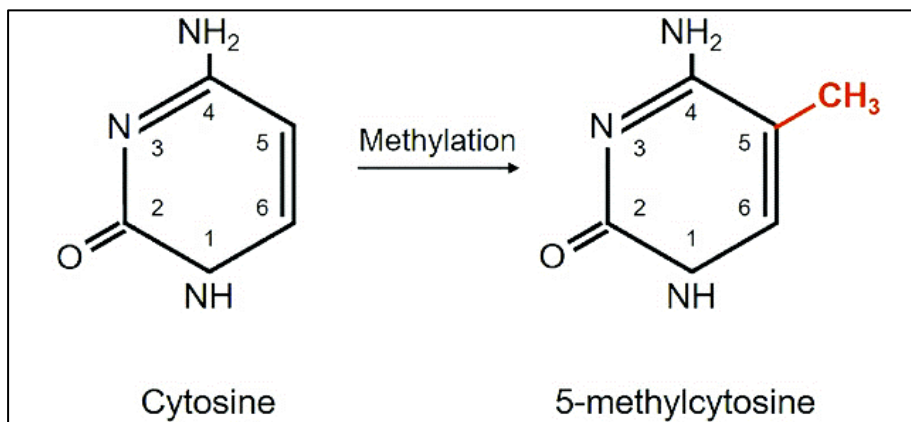
Image adapted from Jovčevska, 2020

DNA methylation is catalysed and maintained through a group of enzymes, knowns as DNA methyltransferases (DNMTs). It is believed that *de novo* DNMTs such as DNMT3a and DNMT3b are involved in establishing early DNA methylation patterns. DNMT1 is also involved in later catalysis and best known to preserve this epigenetic marker (Zhang & Pradhan, 2014). This is done by mimicking and even repairing the original DNA methylation sequence (Hermann et al., 2004; Mortusewicz et al., 2005; Nishiyama et al., 2020).

DNA methylation is distributed all over the genome but commonly aggregate in areas abundant in CpG sites, known as CpG islands and in repetitive sequences in the genome (Bird, 2002; Yong Wang & Leung, 2004). CpG islands are approximately 1kb long and contain clusters of CpG dinucleotides. Repetitive sequences include retrotransposons such as short and long interspersed nuclear elements (SINEs and LINEs) argued to constitute up to 17% of the genome (Lander et al., 2001). The latter are often found to be methylated, whereas CpG island are normally protected from methylation (Suzuki & Bird, 2008). It is

argued that DNA methylation at these sites and across the genome can alter the expression of genes in different ways. One way is through repressing/silencing gene expression at particular sites (Siegfried & Simon, 2010). Another way is through inhibiting the binding of transcription factors to DNA (Attwood et al., 2002).

Control of DNA methylation is essential for normal/typical development and aberrant DNA methylation has been associated with phenotypes such as cancer and X chromosome inactivation (Cotton et al., 2015; C.-J. Lee et al., 2014; Y. Pan et al., 2018; Rodríguez-Paredes & Esteller, 2011; Sproul et al., 2011). There is also emerging evidence associating DNA methylation signatures with neurodevelopmental conditions such as autism spectrum disorder (Andrews et al., 2018; Ladd-Acosta et al., 2014; Loke et al., 2015; C. C. Y. Wong et al., 2014, 2019). There is, however, relatively limited work in the area of psychological distress. In the next section, I review the current literature on DNA methylation and its relationship with indicators of psychological distress (namely, anxiety and depression).

1.7.2. DNA Methylation & Psychological distress

Differentially methylated regions have been associated with anxiety and depression. Research using locus-specific assays indicates several potential candidate genes that are differentially methylated in those with anxiety and depression disorders, including the *BDNF*, *OXTR* and *NR3C1* genes (Chagnon et al., 2015; Klengel et al., 2014; M. Li et al., 2019). Many of these studies report that these epigenetic modifications may be a result of adverse or stressful environments that in turn, affect the physiological processes relevant to the development of anxiety and depressive disorders (Hing et al., 2014). Yet, a candidate gene approach is

unlikely to provide a comprehensive picture of the epigenome especially given that no genes are consistently associated with psychological distress. This points to the importance of hypothesis-free methods as well as expanding to a larger proportion of the epigenome.

Recent advances in technology allows examination of DNA methylation at a genome-wide level. Assays have now been developed to scan over 450,000 CpG loci for DNA methylation status in an exploratory fashion. One such study finds hypermethylation in those with chronic anxiety disorder versus decreased methylation in typically developing individuals (Bortoluzzi et al., 2018). Another epigenome-wide association study (EWAS) finds two differentially methylated regions associated with social anxiety disorder and early life adversity (Wiegand et al., 2021). EWAS results also point to differentially methylated loci in relation to panic disorder (Lurato et al., 2017; Shimada-Sugimoto et al., 2017) as well as depressive disorders and symptoms (Jovanova et al., 2018; Roberson-Nay et al., 2020; Shimada et al., 2018; Starnawska et al., 2019). Although hypothesis-free, EWAS studies are still confined to the CpG loci on the relevant assays/chips used.

In order to gain a global methylomic perspective, DNA methylation has been quantified at repetitive sequences in the genome, including short and long interspersed nuclear elements (SINEs, e.g., Alu and LINEs, e.g., LINE-1). Global DNA methylation has been associated with psychiatric disorders including schizophrenia and bipolar disorder (S. Li et al., 2018, 2019; Murata et al., 2020), as well as with autism spectrum disorder (Tangsuwansri et al., 2018; Tsang et al., 2016). Few studies have explored global DNA methylation in association with anxiety and depression. One study reports higher global DNA methylation in anxious individuals compared to controls (Murphy et al., 2015) and a recent study reports increased

global DNA methylation in Alu and LINE-1 elements in women with depression (Reszka et al., 2021).

Emerging evidence therefore reiterates the importance of DNA methylation in relation to psychiatric disorders and indicators of psychological distress. Nevertheless, studies so far often have small sample sizes and have focused on specific genes or regions. There is also limited work on the role of global DNA methylation in relation to anxiety and depression measures.

1.8. Aims & Structure of this thesis

It is evident that the association between mental and physical health are clearly complex, and there is increasing need to better understand mechanisms behind their comorbidity. The overarching aim of this thesis is to explore the relationship between the two domains under the light of quantitative genetics. Secondly, the aim is to bring this rationale and the twin design to non-western populations, where research still lacks on representation. Our third aim was to explore possible sex differences in these associations. The thesis focuses on widely researched quantitative traits which mainly surrounds anxiety but also extends to traits such as depression, somatic symptoms, and health-related quality of life measures. In light of these aims, this thesis seeks to address the following questions as part of its empirical research section:

1) Is there an autonomic basis to anxiety (chapter 3)?

   This first empirical chapter aims to explore the relationship between cardiovascular

autonomic measures and anxiety symptoms. We use a genetically sensitive twin design to explore: (a) how anxiety symptoms correlate with three cardiovascular autonomic measures (Inter beat-interval, Heart-rate variability and baroreflex sensitivity), (b) the extent to which individual differences in anxiety symptoms and cardiovascular autonomic measures are determined by latent genetic and environmental factors and (3) the genetic and environmental underpinning of the associations between the anxiety-cardiovascular domains.

This is the first study looking at this association using a twin design. A paper has been published based on these results in *Twin Research and Human Genetics* (Nas et al., 2020).

2) How does the relationship between mental-physical health manifest in a non-western setting (chapter 4)?

This chapter uses a Sri Lankan population-based adult twin and singleton sample to investigate a) the genetic and environmental variance components of anxiety symptoms and health related QoL; b) their phenotypic correlations; c) the extent to which overlapping genetic and environmental factors underlie this and d) sex differences in these parameters.

This study is the first in the field exploring the links between anxiety symptoms and health-related quality of life components in a South Asian sample. A paper arising from these findings are published in *Behavior Genetics* (Nas et al., 2021).

3) What is the possible causal mechanism between psychological wellbeing and physical health (chapter 5)?

This third chapter examines whether there is a causal direction in the relationship between physical and psychological health. Using cross-sectional, genetically informative data from the Sri Lankan population-based twin and singleton sample, this causal direction is tested between two latent factors: Psychological distress (Anxiety, Depression, Emotional wellbeing, and Somatic Distress) and Physical health (General health, physical functioning, energy/fatigue, and pain). We tested, in succession, whether a) psychological distress causes decrease in physical health reports, or b) vice versa, c) reciprocal causation or d) no causal links between the two factors.

The chapter adds to the widely researched notion of causality between mental-physical health using a manipulation of the twin method. A paper resulting from this work is currently under review.

4) Is there an epigenetic basis underlying psychological distress (chapter 6)?

This chapter investigates whether there is an association between DNA methylation status and psychological distress. We quantified DNA methylation percentage in the LINE-1 repetitive element in a sample of individuals with autism spectrum disorder and control participants.

This chapter adds to research on non-genetic, epigenetic factors underlying the

occurrence of psychological distress in the population. This work is currently being prepared for publication.

Overall, these research studies shed light on the importance of viewing mental and physical health together rather than as separate entities. They also demonstrate the long-lasting value of the twin design, extensions to it and the relevance in applying this design, more commonly used in western populations, to the rest of the world. Research also points to the role of additional, non-genetic factors in relation to psychological distress such as epigenetic mechanisms which is further explored in the final empirical chapter.

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Chapter 2 . Methods

2.1. Overview

In this chapter, I provide an outline of the samples/cohorts in which the research projects are based on. I then give an overview of the measures used in this thesis. Next, I provide an explanation of the twin methodology, including the relevant twin designs used in this thesis as well as statistical assumptions made. Finally, I provide an overview of epigenetics, with a focus on DNA methylation.

2.2. Samples

This thesis primarily uses twin cohorts to investigate associations between psychological wellbeing, physical health, and health-related quality of life. These samples can also include singletons to increase statistical power. In this section, I outline sample characteristics and selection processes for these cohorts.

2.2.1. Twin Interdisciplinary Neuroticism Study (TWINS)

The Twin Interdisciplinary Neuroticism Study is a three-wave cohort comprising of adult twins, in the northern part of the Netherlands (Riese et al., 2013). Twins were recruited as part of the Groningen Twin registry (GTR). The GTR was formed in 2001, whereby municipalities in the north of the Netherlands (with more than 31,000 inhabitants) were contacted for request of address. Addresses were collected for those individuals born between 1972-1992, from the same mother and sharing the same birth date. Twins (N=1047) identified through this process were invited to take part in the first wave of TWINS (2002). In

2003, wave 2 of data collection was conducted on a sub sample of 125 female twin pairs (N=250). The third wave, conducted in 2006, asked all registered and additionally recruited twins to complete questionnaires, of which 72% returned. In this thesis, we use data obtained in wave 2 (Chapter 3), hence waves 1 and 3 will not be discussed further. The ethics committee at the University Medical Center Groningen approved the study and all participants provided written informed consent.

At wave 2, female twin pairs were invited to a psychophysiological laboratory to complete a series of tasks in an experimental session, as well as collection of additional anthropometric and questionnaire data. In this session, measures on heart rate variability, inter-beat interval and baroreflex sensitivity were collected in four standardised conditions. These measurements began after the participants were in a sitting position for 10 minutes and each condition lasted approximately 5 minutes. Of the 125 twin pairs, 74 (N= 148) were monozygotic and 51 (N= 102) dizygotic. More details on the recruitment of, and conduction of this experimental session is provided elsewhere (Riese et al., 2006, 2007, 2013) and in chapter 3 of this thesis.

Zygosity at wave 2 was determined using 10 microsatellite markers. For three twin pairs, zygosity had to be determined through questionnaire data due to technical failures in DNA genotyping. Exclusion criteria for the study included existing cardiovascular health problems, as well as several criteria to ensure data quality. These are detailed in accompanying supplementary material as part of chapter 3.

2.2.2. Colombo Twin and Singleton Study (COTASS)

The Colombo Twin and Singleton Study is a two-wave population-based study established in the Colombo district of Sri Lanka (Siribaddana et al., 2008). The first wave was conducted between 2005-2007 and the second wave of data collection (COTASS-2) in 2012-2015 (Jayaweera et al., 2018). The study has received ethical approval from Psychiatry, Nursing & Midwifery Research Ethics Subcommittee, King's College London, UK (reference number: PNM/10/11-124), and the Faculty of Medical Sciences University of Sri Jayewardenepura Ethical Review Committee (USJP ERC) (reference number: 596/11).

COTASS is formed as part of the Sri Lankan twin registry, one of the first in the developing world. Twins were identified through birth records, which, although successful in identifying younger twins, was less effective in identifying older twins. A field approach was therefore deemed more appropriate. Taking advantage of annual census visits, a door-to-door survey was used to detect any twins residing in the household, or any twins known to the informant. Twins were excluded if one of the individuals reported that they were not twins, if one or both of the pair had died or gone abroad and if there were no twins at the given address. Out of the 510,835 forms, 66% were returned and the population of twins was determined as 19,302 in the registry. Of this registry, a random sub sample (N=6600) was invited to participate in the COTASS. In the current thesis, we focus on, and ran analyses on this sub-sample. Further details on the full recruitment process can be found elsewhere (Siribaddana et al., 2008).

Twin zygosity was assessed using a cross-culturally adapted and validated questionnaire scored out of 10, given to both twins in a pair (Ooki et al., 1993; Sumathipala et al., 2000). The questionnaire comprised of three questions regarding the degree of similarity of twins (1-3 points); whether they were confused (1-3 points), and if so, by whom (1-4 points). Zygosity was established based on the sum of points from both twins, distributed from 6 to 20. A sum of 6-13 indicated that the twin pair was considered MZ, and if the sum was 14-20, DZ.

2.2.3. Twins' Early Development Study (TEDS)

The Twins' Early development Study is a longitudinal population-based twin study established in the UK. Over 16,000 twin pairs born between 1994-1996 in England and Wales were recruited via national birth records. The sample is representative of the UK population both in terms of ethnicity and socioeconomic factors, as compared with the national averages.

An ongoing study, the first wave of data was collected initially at 18 months old and then at ages 2, 3, 4, 7, 8, 9, 10, 12, 14, 16, 18 and 21. Data collection was conducted through various methods, including postal questionnaire booklets, via telephone, online and at age 21, via a smartphone app. Various incentive are offered to minimise attrition and encourage participation, including small gifts, shopping vouchers and prize draws to reimburse for their time. Over 8000 twins continue to take part in the study. Informed consent was received from parents throughout childhood and directly from the twins at age 16 onwards. Zygosity was determined using a parent-report questionnaire, found to be highly accurate (Price et al., 2000). DNA testing was done when zygosity was unclear.

There are six broad domains of data collected, these cover academic achievement, cognitive development (including language, reading and mathematics), psychopathology (emotional and behavioural development), the environment (school, home, and life events), physical health and wellbeing and finally, personality and motivation. This rich phenotyping has allowed various forms of genetically sensitive analyses to be conducted. More details on the study can be found elsewhere (Rimfeld et al., 2019).

2.2.4. Social Relationships Study (SRS)

The Social Relationships Study was formed as a sub-study of the Twins' Early Development Study, with a focus on individual differences in social and communication skills. A group of twins were selected from the main TEDS sample, who ranged in their social capabilities or difficulties. Some of these individuals have a diagnosis of autism spectrum disorder (ASD) or have high autistic traits, whereas others were selected from TEDS for their good social skills.

SRS has collected in depth behavioural and cognitive measures from individuals across the full range of the autism spectrum. As of date, there has been three waves of data collection, with the first wave conducted in 2007-2011, the second during 2011-2015 and the third throughout 2016-2019. The first wave of SRS established the sample group and in-home testing across the UK was conducted to work with individuals on the autism spectrum, those scoring high and low on autism traits, and their co-twins to assess aspects such as theory of mind, central coherence, executive function, IQ, language skills, mental health, talents and more. The wave collected data from both twins and parents.

The second wave aimed to look at mental health and wellbeing when the twins were 18 years old. Questionnaires were sent out to all SRS twins and parents, enquiring about different aspects of their day to day lives, mental health concerns such as anxiety or depression, as well as education and future plans. The third and most recent phase of SRS focuses on gender differences in relation to social and communication abilities and autism. The measurements used in the study assessed a wide range of factors including theory of mind, mental health, quality of life and wellbeing. More details of the study can be found in previous work (Colvert et al., 2015; Hallett et al., 2013).

2.3. Measures

The current thesis uses a range of self-report and experimental measures to assess psychological wellbeing, indicators of physical health and health related quality of life. Here, I provide an overview of these measures, summarised in **Table 2.1**.

Table 2.1 - Overview of measures used in this thesis

Empirical chapter	Sample	Measure	Measurement
3	TWINS	Anxiety	HSCL + co-twin report (Derogatis et al., 1974)
			POMS (McNair, 1971)
			STAI-DY (Defares et al., 1980; Spielberger, 1989)
		Inter-beat interval	Mean IBI, ms
		Heart rate variability	Power of inter-beat

			intervals in the high frequency band 0.15-0.40 Hz, ms^2
		Baroreflex sensitivity	Gain or modulus, between systolic BP and IBI, in the frequency band 0.07-0.14 Hz, ms/mmHg
4	COTASS	Anxiety	GAD-7 (Spitzer et al., 2006)
		Depression	BDI-II (Beck et al., 1996)
		Somatic distress	BSI (Mumford et al., 1991)
		Emotional wellbeing	SF-36 (Ware & Sherbourne, 1992)
		Role limitations due to emotional problems	
		General health	
		Physical functioning	
		Role limitations due to physical health problems	
		Pain	
		Social functioning	
		Energy/Fatigue	
5	SRS/TEDS	Depression/emotional problems	MFQ (Angold et al., 1995)
			SDQ (Goodman, 1997)

TWINS= Twin Interdisciplinary Neuroticism Study; COTASS= Colombo Twin and Singleton Study; SRS = Social Relationships Study; TEDS= Twins' Early Development Study. HSCL = Hopkin's symptom Checklist ; POMS= Profile of Mood states questionnaire; STAI-DY = State Trait anxiety inventory; IBI = inter-beat interval; BP = Blood pressure; GAD-7 = Generalised anxiety disorder scale ; BDI= Beck's depression inventory; BSI= Bradford Somatic Inventory; SF-36 = Short from health survey; LINE-1 = Long Interspersed Nuclear Element 1; MFQ = Mood's & Feelings Questionnaire; SDQ = Strengths & Difficulties Questionnaire.

2.3.1. Anxiety

For chapter 3, anxiety was measured using a combination of three different questionnaires.

First, a trait anxiety summary score was derived from the Hopkin's Symptom Checklist

(Derogatis et al., 1974). This instrument has been used to capture psychological distress and shortened versions have previously been used in a variety of settings. In this project, we used the 8-item checklist, the HSCL-8, which has been validated in a Scandinavian setting (Fink et al., 1995). The questionnaire asks the following questions: Have you been bothered by any of the following during the last 2 weeks: (1) feeling fearful; (2) nervousness or shakiness inside; (3) feeling hopeless about the future; (4) feeling blue; (5) worrying too much about things; (6) feeling everything is an effort; (7) feeling tense or keyed up and (8) suddenly scared for no reason. The responses were scored from 1 (not bothered) to 4 (very bothered). Four out of the eight items assess anxiety, and a sum score of these items were used in chapter 3.

Anxiety was also assessed using a single-item from the Profile of Mood States questionnaire (McNair, 1971). Participants were asked how anxious they felt over the past week, including on the day of administration. The scores ranged on a Likert scale from 1 (Not at all) to 4 (Extremely). We also used a state anxiety measure from the Dutch adapted version of the State-Trait Anxiety inventory (Defares et al., 1980; Spielberger, 1989). This 20-item sub-scale is scored from 0 (not at all/almost never) to 3 (very much so/almost always). The Dutch adaptation has demonstrated good reliability and validity (van der Bij et al., 2003).

For chapters 4 & 5, anxiety was measured using the 7-item Generalised Anxiety Disorder scale, GAD-7 (Spitzer et al., 2006). The scale captures the presence of core generalised anxiety disorder symptoms by asking how often they have been bothered by the following problems in the past 2 weeks: (1) feeling nervous, anxious, or on edge; (2) being able to stop or control worrying; (3) worrying too much about different things; (4) trouble relaxing; (5) being restless; (6) becoming easily annoyed or irritable; and (**7**) feeling afraid as if something

awful might happen. Scoring is done on a Likert scale, ranging from 0 (Not at all) to 3 (Nearly every day). The scale has been widely used, and has shown to detect anxiety reliably and accurately in the population (Löwe et al., 2008).

2.3.2. Depression / emotional problems

In chapter 5, we used the revised Beck's Depression inventory (BDI-II) (Beck et al., 1996) to assess the presence and severity of depressive symptoms. The 21-item measure asks respondents to self-rate their level of severity on items such as 'Sadness' ranging from 0 (I do not feel sad) to 3 (I am so sad or unhappy that I can't stand it). The measure has been widely used and is found to be a reliable and valid way of examining depression (Dozois et al., 1998; Rodrigo et al., 2015).

We also use the Mood's & Feeling's Questionnaire, MFQ (Angold et al., 1995) in chapter 6 to capture the presence of depression/emotional problems. There are various versions of the original questionnaire, and in the samples that we use, 11 items are selected from the 13-item short version. This short questionnaire asks participants to rate how they might have been feeling or acting in the past two weeks on items such as 'I didn't enjoy anything at all'. Responses were scored on a range, from 0 (Not true), 1 (Quite true) and 2 (Very true). The same items are also used for parents reporting on their children, except that the wording was changed to reflect this, such as 'he/she did not enjoy anything at all'. The MFQ both in its longer and shorter formats, has demonstrated good psychometric properties, including good sensitivity (ability to identify true positives) and specificity (ability to identify true negatives) (Jarbin et al., 2020; Thapar & McGuffin, 1998).

In addition to the MFQ, we also used the Strengths & Difficulties Questionnaire (SDQ) (Goodman, 1997) in chapter 6 to gauge emotional problems. The SDQ is a 25-item measure of psychological attributes, divided into five sections, each measured by five items. These sections are as follows; emotional symptoms; conduct problems; hyperactivity/inattention; peer relationship problems and prosocial behaviour. In this thesis, the focus is on internalising problems, hence the emotional symptoms scale was used. The short scale asks participants to give answers on the basis of how things have been over the last 3 months and consists of items such as 'I am often unhappy, downhearted or tearful'. Items are rated on a 3-point scale, ranging from 0 (Not true), 1 (Quite true) and 2 (Very true). Parents reporting on children had the same items, with the difference in wording, whereby they are asked to give answers on the basis of each child's behaviour over the last 3 months such as 'Often unhappy, downhearted or tearful'. The SDQ is well validated and demonstrates good reliability (Muris et al., 2003; Riso et al., 2010).

2.3.3. Somatic distress

Somatic symptoms, such as tension headaches and heart palpitations, were assessed using the Bradford Somatic Inventory (Mumford et al., 1991). This 21-item questionnaire asks respondents to score the presence of these symptoms in the past month. The participant scores items such as 'have you had severe headaches?' from 0 (Absent), 1 (present on less than 15 days during last month), to 2 (present on more than 15 days during last month). The inventory has shown good psychometric properties across different cultures (Havenaar et al., 1996; Kose et al., 2017).

2.3.4. Health-related quality of life

We used the 36-item short form health survey to assess both psychological and physical health related quality of life (Ware & Sherbourne, 1992). The 36 items examine eight domains: physical functioning (10 items), role limitations due to physical problems (role physical, 4 items), role limitations due to emotional problems (role emotional, 3 items), bodily pain (2 items), social functioning (2 items), emotional wellbeing (5 items), vitality/energy/fatigue (4 items) and general health perceptions (general health, 5 items). An additional item (health transition) is asked but not included in these domains.

The items enquiring role limitations (physical and emotional) use 'yes/no' responses. The other items are scored on a 3-to-6-point category scale. For each item, the raw scores were coded, recalibrated for 10 items, and summed to eight separate domains ranging from 0 -100 whereby 0 equals poorest possible health state and 100 indicating best possible health status. The questionnaire has been used in a variety of settings as a valid and reliable method of in assessing health related quality of life (Bunevicius, 2017; Montazeri et al., 2005; Sullivan et al., 1995).

2.3.5. Cardiovascular functioning

We used three identifiers of cardiovascular functioning, as measured in an experimental task. The first of these was inter-bear interval (IBI), which corresponds to time in between successive heart beats. Second, we measured heart rate variability (HRV), defined as the overall fluctuation of inter-beat intervals and finally, baroreflex sensitivity (BRS) broadly referring to efficiency in regulating blood pressure. Participants were invited to a

psychophysiological laboratory and begun an experimental task after 10 minutes of relaxing

in a sitting position. The task included four standardised conditions: Rest (R1), Stress with

visual feedback (S1), Stress with auditory feedback (S2) and Rest (R2). In the stress

conditions, participants completed a modified version of the 'emotion face dot probe task'

(Mogg & Bradley, 1999).

An ECG was recorded as well as blood pressure and respiration signals being continuously

measured throughout the session. The CARSPAN spectral analysis program (Mulder, 1988;

Robbe et al., 1987) was used to calculate mean IBI (ms), HRV (power of interbeat intervals

in the high frequency band 0.15-0.40 Hz, ms^2) and BRS (gain or modulus, between systolic

BP and IBI, in the frequency band 0.07-0.14 Hz, ms/mmHg). More details on the

experimental and analysis procedure are provided elsewhere (Riese et al., 2006, 2007) as well

as in chapter 3 and its accompanying supplementary material.

2.4. Twin methodology

In this section, I introduce the concept of twinning and its value in quantitative genetics

research. Rooted in biometrical genetics, twin studies play a substantial role in understanding

the genetic and environmental architecture of traits. The classical twin design takes advantage

of the known genetic differences between identical (monozygotic, MZ) and non-identical

(dizygotic, DZ) twins. Monozygotic twins share 100% of their genetic makeup whereas

dizygotic twins share 50% of their genetic material, like any other siblings. Importantly, the

genetic differences refer to the segregating proportion of our DNA which is approximately

1% of the genome (Neale & Maes, 2004; Plomin et al., 2013).

2.4.1. Univariate analysis

The twin design is an excellent resource to separate phenotypic variance (individual differences) of traits into four main sources:

- Additive genetic influences (A), referring to the cumulative effects of individual alleles inherited via parents.

- Non-additive genetic effects (D) represent interactions of alleles at the same locus (dominance) and at different loci (epistasis).

- Common/ shared environmental effects (C) contribute to similarity within family members.

- Unique environmental influences (E) are those exclusive to a family member, causing differences within each other. For example, an accident, different peer groups or differential prenatal exposure of a twin. This also includes measurement error.

The total phenotypic variance in a trait (Vp) is therefore the sum of these components (A+D+C+E). MZ twins share all of their A, C and D influences. In contrast, DZ twins share half of their A influences, all of their C influences, and a quarter of their D effects. Both MZ and DZ twins do not share any of their E influences. This enables us to write a prediction of the twin correlations (or concordances) in terms of these components: The MZ twin correlation (rMZ) is expected to be A+C, whereas the DZ correlation (rDZ) is .5A+C. By comparing correlations on a trait between both types of twins, and solving the equations, we can, thus, estimate the relative contributions of these factors in a twin model.

It is important to note that both D and C influences cannot be estimated at the same time. This is because there are only three statistics (rMZ, rDZ and Vp), and four unknowns (A,C,D and E). Hence, only three sources of influence can be estimated at the same time: either an ACE or ADE model. One way to figure out which model to use is to explore the relative ratios of the twin correlations. Normally, a 2:1 rMZ:rDZ ratio would indicate the role of A, whereas if this ratio is closer to 1:1 this indicates that C is present. If the rDZ correlation is smaller than half the size of the rMZ (e.g., 1: ¼ ratio), this indicates that D is present as D correlates perfectly for MZ twins while only 25% for DZ twins. In this thesis, however, we use the ACE model especially as dominance is rarely seen in twin studies of anxiety. Dominance is therefore not discussed any further.

Taking the ACE model as a basis, standardized A influences are also known as heritability (h^2) defined as the proportion of variance in a trait that can be explained by genetic factors in a population at a given time. This is also known as narrow sense heritability, as the focus is on additive genetic factors (rather than focusing on all genetic factors including dominance and gene-gene interactions). Because DZ twins share, on average, half of their genetic material compared to MZ twins, a rough estimate of heritability is therefore twice the difference in correlation between MZ and DZ twins. This information is used in Falconer's equations, which is further detailed in **Table 2.2**.

<u>Table 2.2</u> - Common equations used in twin model fitting

Equation	Description
$V_p = h^2 + c^2 + e^2$	Standardized phenotypic variance (V=1). Made up of variance by additive genetic factors (h^2), variance due to shared environmental factors (c^2), and variance due to unique environmental factors (e^2).
$R_{MZ} = h^2 + c^2$	Correlation between MZ twins
$R_{DZ} = \frac{1}{2} h^2 + c^2$	Correlation between DZ twins
A or $h^2 = 2(r_{MZ} - r_{DZ})$	Proportion of phenotypic variance explained by genetic factors (heritability)
C or $c^2 = r_{MZ} - h^2$	Proportion of phenotypic variance explained by shared environmental factors
E or $e^2 = 1 - r_{MZ}$	Proportion of phenotypic variance explained by unique environmental factors (also includes measurement error)

MZ = Monozygotic, DZ = Dizygotic, r = Correlation

Path analysis offers a way to analyse twin models and are a useful tool to visualise these models, some of which will be seen in this thesis. Observed variables are represented in rectangles (e.g., Anxiety) and latent variables are represented in circles (e.g., A, C and E influences). Single-headed and double headed arrows represent causal paths and covariance paths, respectively. Covariance between two variables is the sum of all legitimate chains connecting them. The value of a chain is determined by all the traced path coefficients. There are three rules to determine a legitimate chain. Firstly, it is allowed to trace simply forward from variable to variable or trace backwards, then forward. Secondly, tracing twice through the same variable is not allowed. Thirdly, there can only be a maximum of one bi-directional path per chain. We provide an example of a univariate twin model in **Figure 2.1**.

Using these path tracing rules, we can derive the variance of a trait (which is the covariance with itself), which is the sum of all paths from a variable to itself. For example, variance of twin 1 phenotype can be summarised as $Vp1 = a * 1 * a + c * 1 * c + e * 1 * e$, which can also be represented as $a^2 + c^2 + e^2$. Similarly, the covariance between the two twins' can be summed using all legitimate chains. Covariance for MZ twin pairs will therefore be summed and represented as $a*1*a + c*1*c = a^2+c^2$, and for DZ twin pairs as $a*.5*a + c*1*c = .5a^2+c^2$.

Figure 2.1 - Univariate path diagram for a twin pair
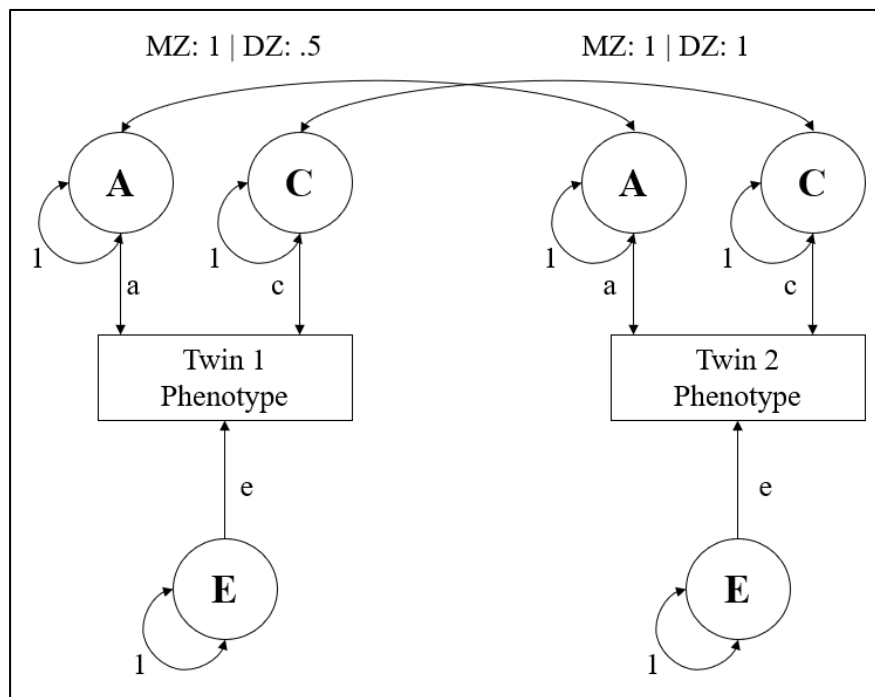
A = Additive Genetic Influences, C = Common Environmental Influences, E = Unique Environmental Influences

2.4.2. Multivariate twin analysis

The univariate twin design can be extended to multiple traits, using multivariate twin designs. These are particularly useful to go beyond phenotypic correlations, decomposing how much of this covariance is due to genetic and environmental effects as well as explore the genetic

and environmental overlap in traits. By comparing MZ and DZ twin correlations, we can estimate the relative contribution of genetic and environmental influences on the variance and covariance of two or more traits. Cross-twin within trait correlations (that is, the correlation between Twin 1 – Twin 2 on a phenotype) are what informs on the variance of a trait. If the MZ:DZ correlation ratio resembles 2:1, we can infer that the variance in the phenotype is likely to be explained by genetic influences, whereas if the ratio is more like 1:1, this would suggest the likely role of common environmental effects.

Similarly, if the cross-twin, cross-trait correlations (that is, the correlation between Twin 1 phenotype 1 – Twin 2 phenotype 2) have a ratio of 2:1, we can infer that the covariance between the two traits is likely to be largely influenced by genetic effects, whereas with a 1:1 ratio, we assume that the covariation is likely to be explained more by common environmental influences. Non-significant cross-twin cross-trait correlations imply that non shared environmental effects are the most likely source of covariance.

There are various ways in which multivariate twin models are specified. One of these is the *Cholesky decomposition*, whereby there are distinct genetic and environmental effects on each variable, as well as paths running from these effects to the other traits. This design is particularly useful in longitudinal twin designs, to be able to parse the genetic and environmental influences overtime. When the order of variables is immaterial, it is more common to present the standardized solution of the Cholesky decomposition, called the *correlated factors solution*, which is primarily used in this thesis (**Figure 2.2**). The correlated factors solution, in addition to distinct genetic and environmental influences on each trait, also includes the respective aetiological correlations between them (e.g., rA = Genetic

correlation, rC = Common Environmental correlation, rE = Unique environmental

correlation). The proportion of phenotypic correlation can be parsed into the relevant ACE

components (Rph due to A, C and E) by multiplying the standardised variance component of

one trait with the aetiological correlation (rA, rC or rE) and then with the standardised

variance component of the second trait. The correlated factors solution is seen in chapter 2.

Figure 2.2 - Correlated factors solution for one individual in a twin pair
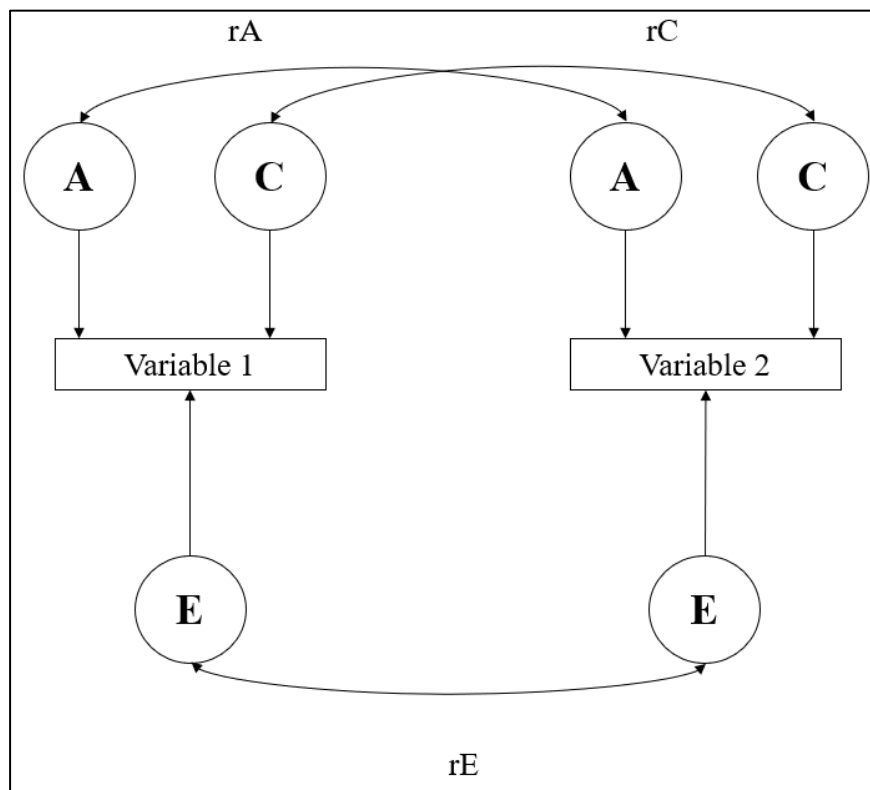
A = Additive Genetic Influences, C = Common Environmental Influences, E = Unique Environmental
Influences. rA = Genetic correlation, rC = Common Environemntal correlation, rE = Unique
environmental correlation.

The independent pathways model is a multivariate twin model, which allows both common

and variable-specific ACE influences. Common influences account for the covariance

between traits, whereas variable-specific influences explain the remaining variance that is not

shared with other traits. This model, however, is not featured in this thesis and will not be

discussed further. Finally, the common-pathways model (**Figure 2.3**), which presumes that

there is a higher-order latent factor with its own unique ACE influences, also referred to as

the 'common' effects. Measured variables load onto this latent factor and have variable-

specific ACE influences. This model is the most parsimonious of the designs mentioned and

is featured in chapters 3 and 5.

Figure 2.3 - Common Pathways Twin Model for one individual in a twin pair
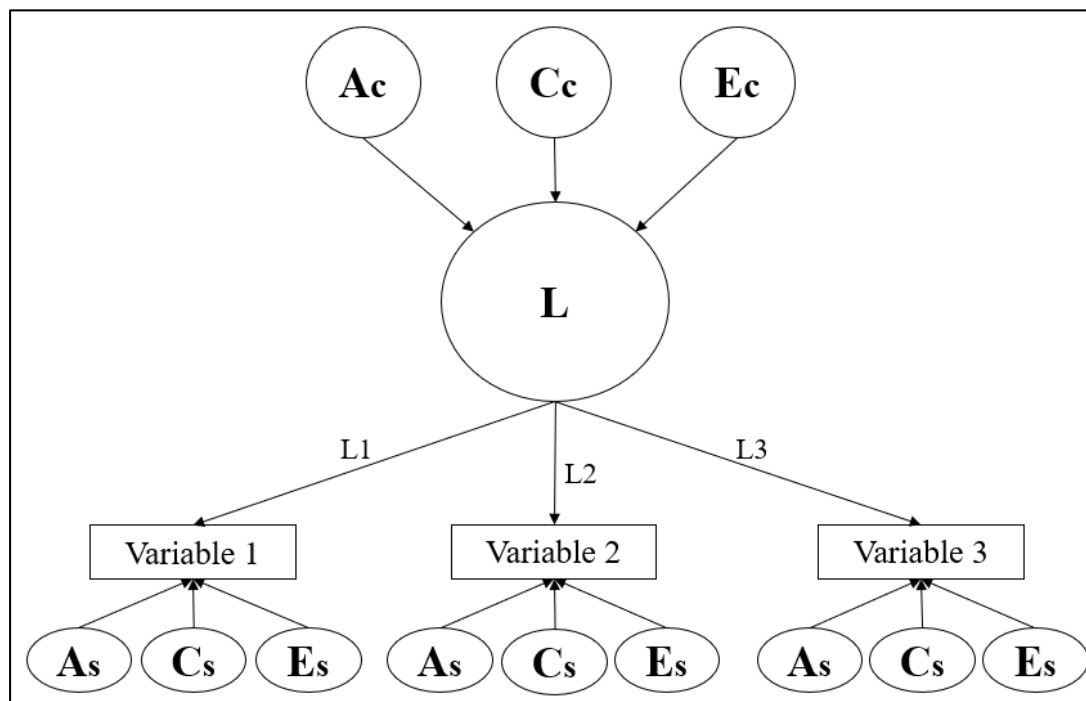
A = Additive Genetic Influences, C = Common Environmental Influences, E = Unique Environmental
Influences. L = Latent common factor. Subscript 'c' and 's' denoting common and specific influences,
respectively. L1-L3 = Loadings from the latent common factor onto measured variables.

2.4.3. Sex differences

It is also possible to explore sex differences in a behaviour genetic context using the twin

design. First, qualitative sex difference models examine whether there are different genetic or

environmental factors underlying variance in a trait across males and females. The power to detect qualitative sex differences comes from DZ Opposite-sex twins, whereby we test if the genetic and environmental correlation between twin 1 (male) and twin 2 (female) can be fixed to .5 and 1 or can be estimated to values between -5 to .5 and -1 to 1 respectively.

Second, quantitative sex differences test whether the magnitude of genetic and environmental effects differ across sex. If there is no deterioration in model fit upon comparing the quantitative with the qualitative models, we conclude that sex differences are likely to be as a result of differences in degree rather than differences in genetic and environmental influences.

Third, the scalar sex difference twin model assumes that there are same standardized ACE influences across sex, and tests if they differ in terms of variance. It is possible to combine this scalar model alongside other forms of sex differences models (e.g., scalar heterogeneity models). All models are also compared to a homogeneity model, whereby aetiology and aetiological correlations are equated across sex. If, the homogeneity model has a significant reduction in fit, we can conclude that the sex differences models are a better fit. Sex differences models are featured in Chapter 2.

2.4.4. Model selection

According to the principle of parsimony, the overarching aim in model fitting is to select the most parsimonious model. Although it is ideal to explain available data with the least number of parameters, model oversimplification can become an issue. The aim is to therefore

select models which do not result in a significant decline in fit if and when it is compared to other, more complex models. In this thesis and in general twin modelling practice, we begin with saturated models, which includes the maximum number of free parameters, estimating means, variances, and covariances of raw data. This model often serves as a foundation, in which later models can be compared with. It is also possible to nest models underneath a more complex one. For example, by comparing a sub-model (e.g., AE or CE model) with an ACE model, we can test whether genetic/ common environmental factors can be dropped from the model without resulting in a significant reduction in fit.

The OpenMx programme (Neale et al., 2016) is a package in R (https://www.r-project.org/) (R Core Team, 2017), widely used by the statistical genetics community. This statistical package is used to analyse twin data in the current thesis. The package uses matrix algebra and the observed variance-covariance matrices and runs on maximum-likelihood estimation. The main fit statistic to determine best-fitting models is the minus twice the log likelihood (-2LL) of observations. The differences in -2LL can also be represented as χ^2 distributions and fit of sub-models can therefore be examined by χ^2 difference tests. Lower values of -2LL indicate a better fit.

In addition to -2LL, a range of fit indices can be used to gauge best-fitting models. Of these, AIC (Akaike's Information Criterion) and BIC (Bayesian Information Criterion) indices are used to identify best-fitting models in this thesis. Lower (more negative) values indicate better fit on both these criteria. AIC differences that are less than 2 suggests substantial evidence for the model that is more parsimonious, AIC differences between 3-7 suggests considerably less support for the higher AIC model and differences larger than 10 indicate

that the lower AIC model is a better fit (Burnham & Anderson, 2002). For BIC, differences of 1-2 indicate weak evidence for model distinction, 2-6 indicating positive, 6-10 strong and >10 suggesting very strong evidence for selecting the model with lower BIC(Raftery, 1995).

2.4.5. Assumptions Of The Twin Design

Like many other statistical methods, there are certain assumptions that need to be considered with the twin design. These assumptions are listed below alongside methods to verify whether these are violated.

- *MZ and DZ twins do not differ in terms of their shared environmental exposure.*

This is widely known as the equal environments assumption (EEA). Violation of this assumption, such that MZ twins are actually treated more similarly and share more of their environment than DZ twins (Plomin et al, 2013; Fosse, Joseph & Richardson, 2015), can inflate MZ twin correlations and thus overestimate genetic influence on traits. On the other hand, if DZ twins are treated more similarly than MZ twins, this could inflate the effect of the shared environment.

There are many ways in which both types of twins share similar environments, including their prenatal environment, family rearing and schools attended. The former, however, can also introduce possible differences, whereby MZ twins often share their chorion during pregnancy (protective foetal membrane), whereas DZ twins are exclusively dichorionic. This may mean that MZ twins actually share their prenatal environment to a larger extent. The effects of chorionicity on heritability estimates, however, are not replicated and results

remain largely inconclusive or limited to a small number of phenotypes (Beijsterveldt et al., 2016; Marceau et al., 2016).

To examine the EEA further, research has looked to mislabelled twin similarities. If MZ twins are indeed treated more similarly, then mislabelled DZ twins should be more alike. In contrast, mislabelled MZ twins should be less alike. Research, however, has found that this mislabelling had little to no influence on twins' similarity on complex behavioural traits (Conley et al., 2013; Kendler et al., 1993). It is also argued that MZ twins may be in more frequent contact with each other over the lifespan, share more childhood experiences and other social networks (e.g., friendship groups). Again, these shared experiences and contact, though, do not significantly impact phenotypic resemblance on trait (LoParo & Waldman, 2014). It is therefore clear that although the EEA may not always hold, the possible bias that could be introduced is not significant and can have an influence on both MZ and DZ twin correlations.

- *There are little/no gene environment correlations and interactions for the trait under investigation.*

Twin modelling also assumes that gene-environment correlations and interactions (genetic control over sensitivity to environments) are minimal. Gene-environment correlations (rGE) refer to genetic influence on exposure to specific environments. There are three types: passive, active and evocative rGE. A passive rGE refers to the correlation between the genes and environments that parents pass to their children. For example, children with extraversion tendencies also more likely to be raised in chaotic home environments (Lemery-Chalfant et al., 2013). Active rGE occurs when individuals' select environments that correlate with their

genotype. For instance, someone with a genetic disposition for anxious personality may be socially avoidant. An evocative rGE occurs when individuals' evoke a response from their environments based on their genetic propensities. For instance, children with high levels of anxiety may elicit a controlling parenting style (Eley et al., 2010). There are several lines of evidence supporting the role of rGE, with many environmental measures such as stressful life events, divorce and trauma showing moderate heritability (Kendler & Baker, 2007; Perlstein & Waller, 2020). In terms of twin modelling implications, a positive rGE will increase, whereas a negative rGE will decrease estimates of genetic components (Rijsdijk & Sham, 2002).

Another form of gene-environment interplay, gene-environment interactions (GXE) refer to sensitivity to particular environmental factors based on one's genotype. The phenomenon has attracted research attention particularly for stress and anxiety related disorders (Sharma et al., 2016). Early studies have often focused on particular genetic markers, such as the serotonin transporter gene (5-HTT), although later studies have failed to replicate this effect and/ or have shown very small effects (Bleys et al., 2018).

Due to the aims and nature of our analyses, we did not investigate gene-environment interplay. The twin model does not cope well with gene-environment interplay and its detection is often difficult (Rijsdijk & Sham, 2002). If there is a gene x non-shared environmental interaction, this could inflate the role of the non-shared environmental component as both MZ and DZ twin correlations would decrease. If, however, there is a gene x common environmental interaction, this could result in an overestimation of the genetic component (as both twins share 100% of their common environment but differ in their

genetic sharing, there is a higher chance of interactions with the MZ twins). Gene—

environment interplay is therefore an important consideration, though can have bi-directional

influences on estimates depending on which component of the environment interacted with.

- *There is no assortative mating.*

Assortative mating refers to the non-random selection of reproductive partners. This sexual

selection can affect twin modelling estimates, as more genes and environments would be

shared by partners and ultimately by their children. Hence, DZ twins may actually share more

than 50% of their segregating DNA, resulting in inflated C estimates in twin models. Several

studies suggest that assortative mating is evident, though the impact of this is found to be

negligible (Maes et al., 1998; Robinson et al., 2017). Phenotypic correlations between parents

for a trait and tracking partner resemblances overtime can help to detect this effect further.

- *Twins are representative of the general population.*

The twin model also rests on the idea that twins are representative of the general population.

Twins, however, can differ from singletons on various occasions, particularly surrounding

their prenatal environment, obstetric complications and mortality (Papiernik et al., 2010).

These differences can contribute to differences in development and prevalence of psychiatric

symptoms. These factors, however, often do not play a substantial role and is limited to a few

traits (Andrew et al., 2001; Beijsterveldt et al., 2016). In addition, prevalence and mean

scores of common psychiatric symptoms is not found to significantly differ across twins and

singletons (Kendler et al., 1995). Overall, twins do not seem to differ from singleton

comparisons consistently and significantly, making the assumption generally valid.

Taken together, although the assumptions of the twin design are not always held, these limitations are unlikely to have a significant or large impact on estimates obtained.

Additionally, violations of these assumptions can both inflate/deflate estimates in opposite directions, hence any effect(s) could essentially cancel each other out. It is, nevertheless, important to note that estimates obtained from twin modelling are not definite and should be interpreted under the light of these presumptions. The advantages of the twin design therefore outweigh potential limitations and biases, although results from other study designs should be used to form a better understanding of traits explored.

2.5. DNA Methylation

This thesis also incorporates analysis of DNA methylation, as a measure of epigenetics, introduced in chapter 1. We gauged global DNA methylation in this thesis (chapter 6) by quantifying level of DNA methylation at repetitive elements in the genome (Pappalardo & Barra, 2021; Yang et al., 2004; Zheng et al., 2017). Repetitive elements such as transposons constitute approximately 45-50% of the human genome (Lander et al., 2001; Lisanti et al., 2013), making it an ideal alternative target to explore global DNA methylation. In this thesis, we focus on the repetitive Long Interspersed Nuclear Element-1 (LINE-1). The LINE-1 assay has been shown to be the best possible surrogate over and above other assays, obtaining close estimates to those through gold standard methods (Lisanti et al., 2013). Here, I introduce methodological details of this procedure, including sodium bisulfite conversion, polymerase chain reaction (PCR) protocol and the quantification of DNA methylation.

2.5.1. Sodium Bisulfite Conversion

In order to identify methylated cytosine bases, a conversion process was carried out using sodium bisulfite ($NaHSO_3$). This process relies on the chemical transformation of unmethylated cytosine bases to uracil when treated with sodium bisulfite which are replaced by thymine bases following PCR amplification. Methylated cytosine bases, on the other hand, remain unchanged. In this thesis, bisulfite conversion was conducted using a well validated, widely used kit, the EZ-96 DNA Methylation-Gold ™ Kit (Zymo Research, CA, USA). We followed the manufacturer's protocol to conduct bisulfite conversion. **Figure 2.4** illustrates the bisulfite conversion effect on original DNA sequence with both methylated and unmethylated cytosines.

Figure 2.4 - DNA sequencing results following bisulfite conversion
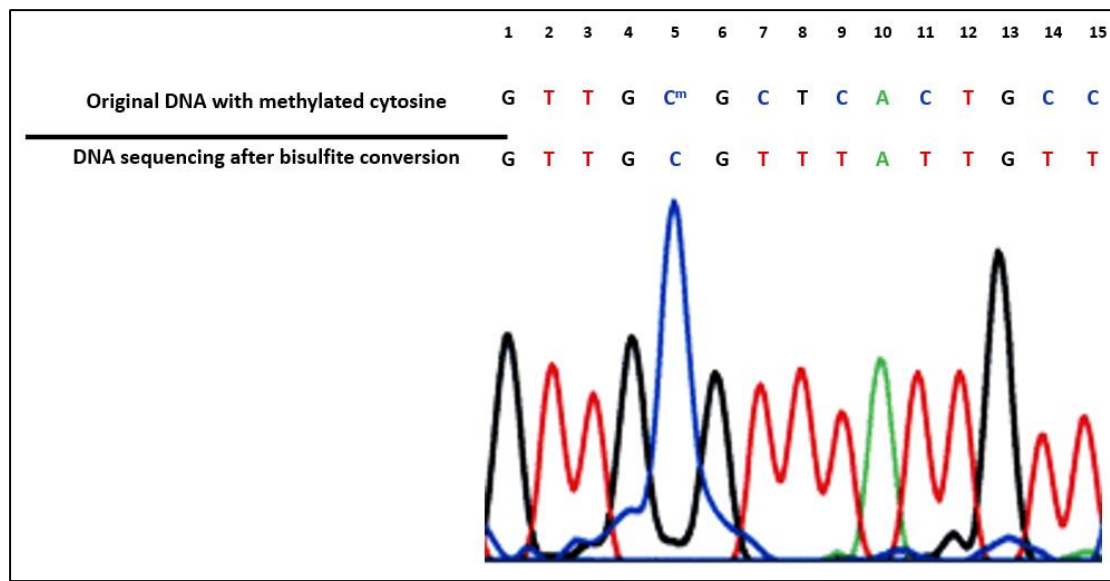
Genomic DNA with methylated cytosine (at nucleotide position 5) and unmethylated cytosines (at nucleotide positions 7, 9, 11, 14 and 15) was bisulfite treated and the recovered DNA was PCR amplified and then sequenced directly. The methylated cytosine remains the same whereas the unmethylated cytosines were converted into uracil following bisulfite treatment and detected as thymine bases following PCR (figure adapted from Zymo research's EZ-96 DNA Methylation kit).

2.5.2. Polymerase chain reaction (PCR)

Polymerase chain reaction is a method to target genomic regions of interest, by amplifying short segments of DNA to produce large quantities of specific sequences lying between two regions of known DNA. In this thesis, we amplified the LINE-1 repetitive region in DNA using primers based on previous literature (Guarrera et al., 2015; Wang et al., 2010). Primers were designed using the online Agena Bioscience EpiDesigner software (Agena Bioscience Inc, CA, USA; http://www.epidesigner.com) and BiSearch (http://bisearch.enzim.hu/; (Tusnády et al., 2005)). Primer sequence and CpG site coverage were validated using the RSeqMeth package in R (https://cran.r-project.org/src/contrib/Archive/RSeqMeth/). This ensures that the target sequence covers the CpG sites of interest and are not overlapping with other CpG sites. **Table 2.2** provides details on the forward and reverse primers for the LINE-1 assay. More details on this methylomic measure and relevant experimental protocol are outlined in chapter 6.

Table 2.3 - Details on the LINE-1 assay

Name of primer	Use	Forward primer	Reverse primer
LINE-1	Estimating Global DNA methylation	aggaagagagGTGTGAGG TGTTAGTGTGTTTTG TT	cagtaatacgactcactatagggagaag gctATATCCCACACCTAAC TCAAAAAAT

LINE-1 = Long Interspersed Nuclear Element -1. The single letters denote nucleotide bases in the genome whereby: A/a = adenine; G/g = Guanine; C/c = Cytosine; T/t = Thymine.

We used a standard protocol to conduct a 10 μl PCR mix, details of this are provided in **Table 2.3**. all PCRs performed in this thesis were amplified using hi-fidelity hot start *Taq* (Qiagen Hot Start *Taq* Polymerase), which becomes active at 95°C and is known to reduce

mispriming and primer-dimer formation. The PCR mixtures were then placed in a thermal

cycler which operates on the following cycling program:

Step 1: 94°C for 4 mins

Step 2: 94°C for 20 seconds

Step 3: 56°C for 30 seconds

Step 4: 72°C for 1 minute

Step 5: Step 2, 44 times

Step 6: 72°C for 3 minutes

Step 7: 4°C for 10 minutes

TOTAL RUNNING TIME ~ 2hrs 24 mins

Table 2.4 - Polymerase chain reaction mix

Reagent	Volume, 1 Reaction (µl)
10X Buffer	1
MgCl₂	0.2
dNTPs mix (25 mM each)	0.08
F primer	0.4
R primer	0.4
Hot Start Taq polymerase (5U/µl)	0.08
H₂O	5.84
DNA	2
TOTAL	10

2.5.3. Agarose Gel Electrophoresis

Once PCR is completed, a standard procedure to check if PCR has amplified the DNA is by

running the products on a gel electrophoresis. Amplified DNA is visualised in the gel through

the addition of ethidium bromide, which binds strongly between DNA bases, and its

fluorescence allows absorption of invisible UV light, transmitting the energy as visible orange light. The majority of agarose gels are made between 0.7% and 2%. A 0.7% gel shows good resolution of large DNA fragments 5–10kb and a 2% gel shows good resolution for small fragments (0.2–1kb). In this thesis, we used 1% gels and were made using the following protocol:

1) 1g agarose added to 100ml 1X TBE buffer.

2) Mix heated in a microwave for ~1 minute until agarose is completely dissolved in buffer.

3) Cooled for ~5 minutes until it reaches ~60°C.

4) 1µl Ethidium Bromide (10mg/ml) added and mixed well.

5) Gel poured slowly into gel tank and combs added.

6) Gel left to set for ~45 minutes.

7) 1X TBE buffer poured into the gel tank to submerge the gel to 2–5mm depth.

To check amplification, approximately 5µl of PCR product was run in each gel lane along with ~3µl of Orange G loading Buffer. A DNA size marker/ladder (ΦX174) was run on each gel so that fragment sizes could be accurately estimated. Gels were usually run at ~110v for ~1 hour, although specific running conditions varied depending on the products being examined. Once run, gels were analysed under UV light. An example gel electrophoresis run is featured in **Figure 2.5** with DNA samples moved away from the original gel wells, signalling amplification of DNA.

Figure 2.5 - Example gel electrophoresis result from a test run

ΦX174 Ladder — Samples — PCR Mix + H₂0 — ΦX174 Ladder
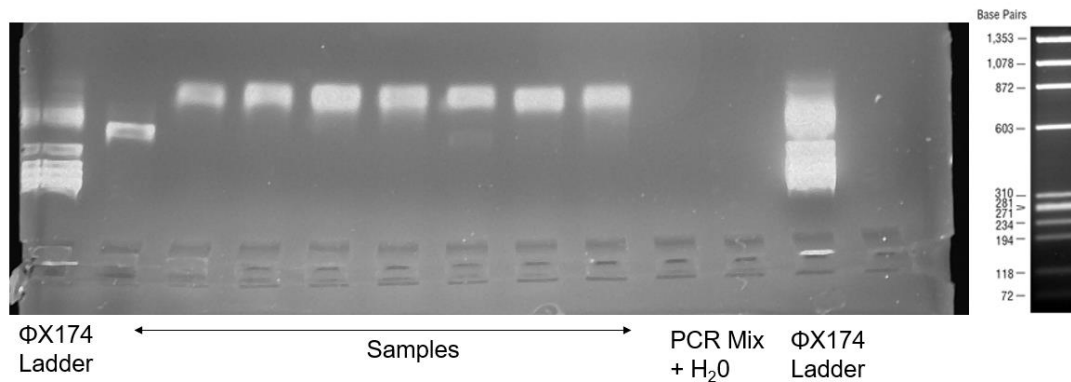
2.5.4. Quantifying DNA methylation

Following gel electrophoresis, amplified DNA underwent SAP and massCLEAVE reactions. The shrimp alkaline phosphatase (SAP) reaction dephosphorylates any remaining unincorporated nucleotides in the amplified products. Next, a massCLEAVE reaction is performed to cleave the DNA specifically at the T nucleotide bases (van den Boom & Ehrich, 2007). This transcription reaction is performed with a special nucleotide mix, which leads to T-specific cleavage with the addition of RNAse A enzyme. Next, we performed a conditioning stage, whereby DNA is mixed with resin to remove salts from the phosphate backbone of the DNA cleavage products.

Following this, DNA was dispensed onto a spectroCHIP® array using a nanodispenser (nanodispenser RS1000) at a volume of 120mm/sec. The spectroCHIP® was then loaded onto the Agena Biosciece EpiTYPER MassARRAY® platform to analyse the level of DNA methylation of the samples (Suchiman et al., 2015). Fully methylated samples were also included as positive controls in the experiment. The mass spectrometry (MS) system uses a Matrix-Assisted Laser Desorption Ionization – Time of Flight (MALDI-TOF) technique for

the precise detection of DNA methylation (Ehrich et al., 2005). The matrix on the SpectroCHIP® absorbs the energy of the laser and transfers it to the DNA fragments which subsequently become ionized. The ionized fragments are separated by the time it takes to arrive at the detector in at the end of the mass spectrometer's vacuum chamber. The higher the mass, the longer it takes for the time of flight.

If a CpG dinucleotide is methylated and hence protected from bisulfite conversion, the corresponding DNA fragment, the CpG unit, will be 16 Da (Dalton) heavier in mass. This results in a 16 Da shift in the mass spectrum. This difference in mass is therefore what differentiates methylation status in the DNA strand. Finally, the EpiTYPER software was used to analyse and visualise this data as well as export the data for further analyses. The full procedure of quantifying DNA methylation is outlined in **Figure 2.6**.

Figure 2.6 - Overview of Agena Bioscience EpiTYPER process
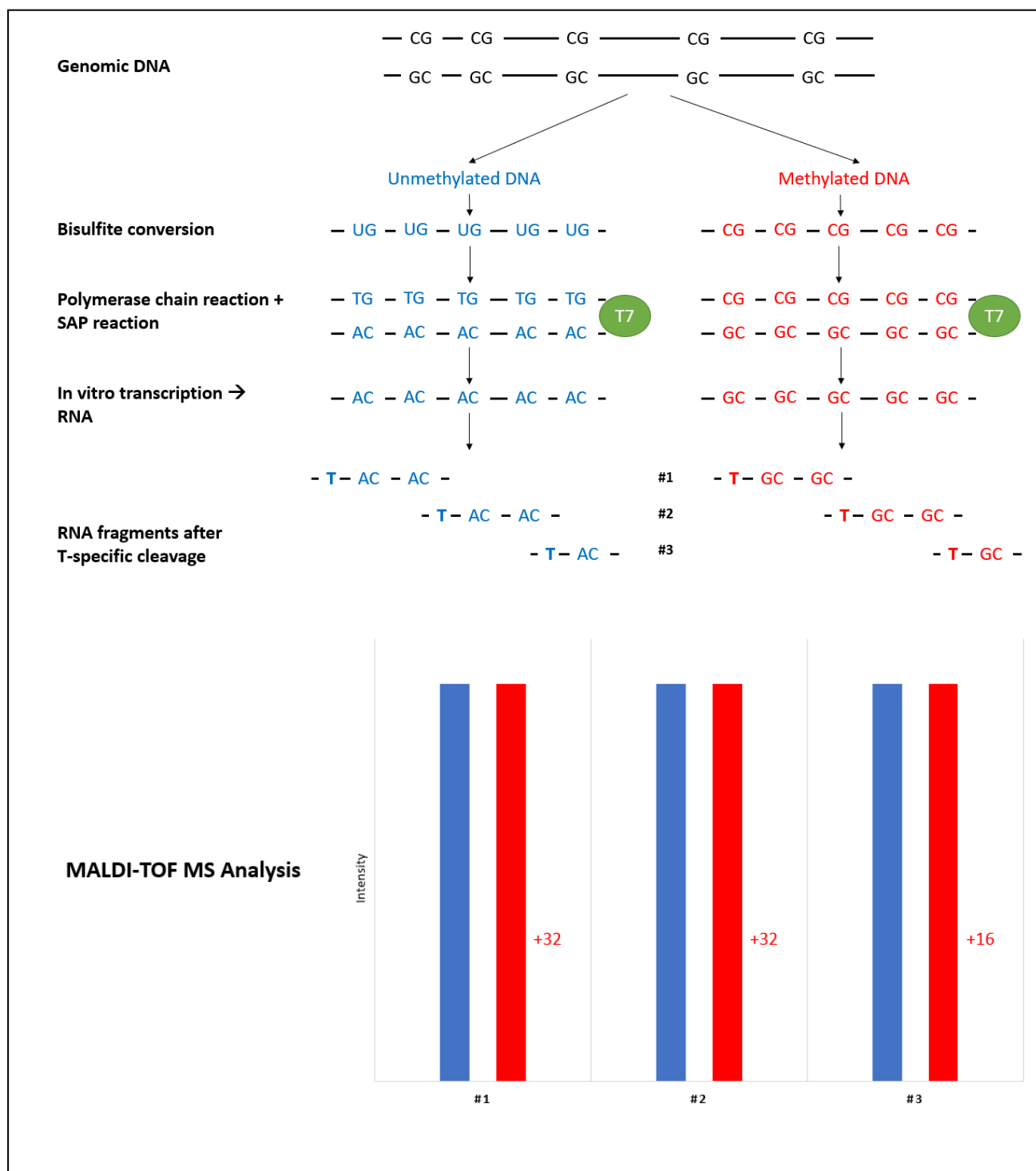
T7 = T promoter; MALDI-TOF MS = Matrix-Assisted Laser Desorption Ionization – Time of Flight Mass Spectrometry. Figure adapted from Agena Bioscience EpiTYPER information brochure.

2.5.5. References

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Chapter 3 . Higher Anxiety Is Associated with Lower Cardiovascular Autonomic Function in Female Twins

This chapter is an accepted manuscript version of a peer-reviewed publication:

Supplementary material is provided at the end of the chapter

Higher Anxiety Is Associated With Lower Cardiovascular Autonomic Function In Female Twins

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Running title: Anxiety And Cardiovascular Autonomic Dysfunction

Word count: 3870

Number of figures: 2

Number of tables: 5

Abstract

Anxiety symptoms co-occur with cardiovascular health problems, with increasing evidence suggesting the role of autonomic dysfunction. Yet, there is limited behaviour genetic research on underlying mechanisms. In this twin study, we investigated the phenotypic, genetic and environmental associations between a latent anxiety factor and three cardiovascular autonomic function factors; inter-beat interval (time between heart beats), heart rate variability (overall fluctuation of heart-beat intervals) and baroreflex sensitivity (efficiency in regulating blood pressure). Multivariate twin models were fit using data of female twins (N=250) of the Twin Interdisciplinary Neuroticism Study (TWINS). A significant negative association was identified between latent anxiety and baroreflex sensitivity factors ($r = -.24$, 95% CI = -.40, -.07). Findings suggest that this relationship was mostly explained by correlated shared environmental influences, and no evidence for pleiotropic genetic or unique environmental effects. We also identified negative relationships between anxiety symptoms and heart rate variability ($r = -.17$, 95% CI= -.34, .00) and inter-beat interval factors ($r = -.13$, 95% CI= -.29, .04), though these associations did not reach statistical significance. Findings implicate that higher anxiety scores are associated with decreased efficiency in short-term blood pressure regulation, providing support for autonomic dysfunction with anxiety symptomatology. The baroreflex system may be a key mechanism underlying the anxiety-cardiovascular health relationship.

Keywords: Anxiety, Cardiovascular, Autonomic dysfunction, Baroreflex sensitivity, Inter-beat interval, Heart rate variability.

Introduction

Anxiety symptoms are common, and if excessive in magnitude, duration and frequency, can lead to clinical anxiety (Mallorquí-Bagué, Bulbena, Pailhez, Garfinkel, & Critchley, 2016; Mehta et al., 2003). Both symptomatic and clinical anxiety co-occur with cardiovascular health problems (Allgulander, 2016; Celano, Daunis, Lokko, Campbell, & Huffman, 2016; W. H. Chang et al., 2016; Emdin et al., 2016; Janszky, Ahnve, Lundberg, & Hemmingsson, 2010; Pratt, Druss, Manderscheid, & Walker, 2016; Vogelzangs et al., 2010). Longitudinally, anxious individuals have an elevated risk of coronary heart disease (CHD), independent of demographic variables (e.g. age), biological risk factors (e.g. family history) and lifestyle/health behaviours (e.g. exercise) (Roest, Martens, de Jonge, & Denollet, 2010). Somatic symptoms of anxiety, such as heart palpitations, are also associated with an increased CHD risk in women (Nabi et al., 2010), highlighting a physiological, autonomic pathway in which anxiety may link to cardiovascular events (Celano et al., 2016).

Autonomic dysfunction, an imbalance between parasympathetic and sympathetic control, may contribute to this cardiovascular burden. To test this, cardiovascular autonomic functioning has been measured using three indices: baroreflex sensitivity (BRS), inter-beat interval (IBI) and heart rate variability (HRV). BRS reflects efficiency in responding to blood pressure (BP) changes. Short term regulation of BP is achieved through baroreceptors, which detect an increase in BP, resulting in a reduction of heart rate through inhibition of sympathetic activity and activation of parasympathetic flow. The inverse occurs when BP is decreasing (Shaffer, McCraty, & Zerr, 2014; Swenne, 2013). There is limited research on the anxiety-BRS relationship and existing studies mainly take a clinical perspective (Mussgay & Rüddel, 2004).

Nonetheless, anxiety symptoms have been associated with lowered BRS by up to 36%, independent of demographic variables and existing cardiovascular health predictors (Virtanen et al., 2003; Watkins, Blumenthal, & Carney, 2002; Watkins, Grossman, Krishnan, & Sherwood, 1998). The association is apparent over and above depression (Watkins, Grossman, Krishnan, & Blumenthal, 1999) and recorded in response to stress, argued to induce a shift in autonomic reactivity (Ginty, Kraynak, Fisher, & Gianaros, 2017). Following a stress-inducing task, young adults scoring high on trait anxiety display lower BRS compared to their low trait anxiety peers (Sanchez-Gonzalez et al., 2015). Furthermore, this highly anxious group had an attenuated BRS comparable to a middle-aged sample, suggesting cardiovascular outcomes similar to that produced with ageing. Anxiety symptoms may therefore associate with reduced parasympathetic activity both at baseline and in response to stress.

Varying IBIs, the period in between successive heart beats, is a marker of healthy cardiovascular autonomic functioning (Costa, Davis, & Goldberger, 2017). Typically measured as the time between 'R' peaks on an electrocardiogram, IBI is also referred to as the 'RR interval'. Clinical anxiety studies suggest shorter IBIs, indicative of increased heart rate with low variability (Hoehn-Saric, McLeod, & Zimmerli, 1991; Thayer, Friedman, & Borkovec, 1996).

Heart rate variability (HRV) can be defined as the overall fluctuation of heart period over time (Chalmers, Quintana, Abbott, & Kemp, 2014). HRV can be indexed using several methods, including based on IBI time series (e.g. mean IBI, mIBI), or frequencies (Low;

LF$_{IBI}$, 0.04 – 0.15 Hz and high; HF$_{IBI}$, 0.15 – 0.40 Hz) and non-linear techniques (e.g. Poincaré plots). Being a common marker of psychological well-being, cardiovascular health and mortality (Chalmers et al., 2014; Kemp & Quintana, 2013), HRV has been negatively associated with anxiety phenotypes. For example, HRV as measured in short time periods in the high-frequency band have been associated with generalised anxiety (H.-A. Chang et al., 2013b; Yeragani, Tancer, Seema, Josyula, & Desai, 2006), panic disorder (H.-A. Chang et al., 2013a; Wang et al., 2013) and social anxiety (Gaebler, Daniels, Lamke, Fydrich, & Walter, 2013; Pittig, Arch, Lam, & Craske, 2013). As with BRS and IBI studies, research focus is on clinical anxiety which does not represent its dimensional, quantitative nature in the population (Bjelland et al., 2009; Kircanski, LeMoult, Ordaz, & Gotlib, 2017).

There is also sparse research on what underlies this autonomic dysfunction. According to neurobiological models (Friedman 2007; Thayer and Lane 2000), anxiety reflects poor inhibition of cognitive (e.g. worry), affective (e.g. panic), behavioural (e.g. avoidance), and physiological (e.g. increased heart rate) responses, reducing autonomic and physiological flexibility (Thayer, Yamamoto, & Brosschot, 2010). Sex differences have also been reported, whereby women show decreased parasympathetic activity in comparison to men (Fiol-Veny, De la Torre-Luque, Balle, & Bornas, 2018; Koenig, Rash, Campbell, Thayer, & Kaess, 2017), though findings are inconclusive, with other studies indicating increased heart variability in women (Snieder, van Doornen, Boomsma, & Thayer, 2007). There is also, limited behavioural genetic research on the common genetic (pleiotropy) and environmental influences that could link the two domains as they appear in the normal population. Twin studies are imperative in understanding this relative contribution to individual differences in traits, as done with anxiety symptoms previously (Ask, Torgersen, Seglem, & Waaktaar,

2014; López-Solà et al., 2014; Nivard et al., 2015; Petkus, Gatz, Reynolds, Kremen, & Wetherell, 2016). Previous studies, using the same sample as used here, suggests genetic influences on BRS, HF_{IBI} and mIBI, plus on the relationship between neuroticism and BRS (Riese et al., 2006, 2007). Yet, these parameters have not been investigated in the context of anxiety symptoms.

This behavioural genetics study uses a genetically sensitive twin design to explore i) how anxiety *symptoms* correlate with *the three* cardiovascular autonomic measures (mIBI, HF_{IBI}, BRS) ii) the extent to which individual differences in anxiety symptoms and cardiovascular autonomic measures are determined by latent genetic and environmental factors and iii) the genetic and environmental underpinning of the associations between the two domains.

Materials and Methods

Participants

This study capitalises on the Twin Interdisciplinary Neuroticism Study (TWINS) (Riese, Rijsdijk, Snieder, & Ormel, 2013), of the Groningen Twin Register (GTR) in the north of The Netherlands. Monozygotic (MZ) and dizygotic (DZ) twins were identified for the GTR based on being born between 1972-1992 from the same mother with identical birth dates. In the current study, we used data from a subset of female twin pairs (N=250) aged 18-30 who participated in a laboratory session as part of TWINS. Individuals with existing cardiovascular health problems were not considered for inclusion of the study. The study was given ethical approval by the ethics committee at the University Medical Center Groningen,

and all individuals provided written consent (METc 2000/060e).

Measures

Anxiety symptoms

We included four measures of anxiety symptoms for each twin. This included a mix of both state and trait measures to gauge an overall anxiety symptoms composite. First, a trait anxiety sum score was derived from 4 items in the 8-item Hopkins' Symptom Checklist (HSCL) a validated psychometric tool to measure general psychological distress (Derogatis, Lipman, Rickels, Uhlenhuth, & Covi, 1974). Second, a single item state anxiety score measured by the Profile of Mood States (POMS) questionnaire (McNair, 1971). Third, a single-item state anxiety measure from the Dutch version of the state-trait anxiety inventory (STAI-DY) (Defares, Ploeg, & Spielberger, 1980). Finally, we included the HSCL anxiety sum score from the co-twin sister, also derived from four items in the HSCL (Twin 1 reporting on Twin 2 and vice versa). This is done to control for self-report bias and decrease variance in self-reported mental health (Kendler, Prescott, Jacobson, Myers, & Neale, 2002; Riese et al., 2007). All measures were treated as continuous, except for the POMS variable which was entered as a dichotomous variable (as it is originally ordinal data).

Cardiovascular Autonomic Functioning

Participants were instructed to abstain from intensive physical activity (including sports) and alcohol consumption 24 hours before testing and to fast (including coffee and tea consumption) from 10:00 pm on the evening before visiting the lab. Measurement of cardiovascular autonomic functioning has been described in detail elsewhere (Riese et al., 2007, 2013) and outlined in **Supplementary material 1**. Briefly, BRS, HRV and mean IBI

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were measured in an experimental task with four standardised conditions; Rest (R1), stress with visual feedback (S1), stress with auditory feedback (S2) and rest (R2). Participants completed the tasks in a seated posture. In the stress conditions, participants completed a modified version of the 'emotion face dot probe task' (Mogg & Bradley, 1999), whereby participants indicate whether they saw three/ four dots previously occupied by a pair of faces. Visual feedback was presented as the correct answer at the centre of the screen for 1000 ms. In the auditory feedback condition, participants' wrong answers were met with 100dB of white noise for 500 ms.

Cardiovascular measurements were collected in a sitting position after participants relaxed for ten minutes, with each condition lasting approximately five minutes. An electrocardiogram (ECG) was recorded using Ag/AgCl electrodes (3M^{TM} Red Dot^{TM,} St Paul, MN, USA) and a custom-made ECG-amplifier and trigger device (ETC-3, DataLab, Faculty of Behavioural and Social Sciences, University of Groningen, The Netherlands). A Portapres device continuously measured beat-to-beat blood pressure from the finger (FMS Finapres Medical Systems BV; Amsterdam, The Netherlands). As respiration is known to influence BRS, changes in respiration signals were recorded with a flexible band placed on the upper thorax connected to an amplifier. ECG, finger blood pressure and respiration were digitized using a data acquisition board (Keithley DAS-12, Keithley Instruments, Inc., USA) at 100 Hz. Custom-made PreCar 3.0 (Greaves-Lord et al., 2010) software was used for R-peak detection (at ±2 ms accuracy) and artefact correction (i.e. IBI time-series with supraventricular extra systoles were excluded).

mIBI (mean of the inter-beat intervals, ms), HF$_{IBI}$ (Power of inter-beat intervals in the high

frequency band 0.15-0.40 Hz, ms²) and BRS (gain or modulus, between systolic blood pressure and inter-beat interval, in the frequency band 0.07-0.14 Hz, ms/mmHg) were calculated using the CARSPAN spectral analysis program (Mulder, 1988; Riese et al., 2007; Robbe et al., 1987), a method that has been previously used (Althaus et al., 2004; Dietrich et al., 2006; Lefrandt et al., 1999; Van Roon, Mulder, Althaus, & Mulder, 2004). More details on exclusion criteria can be found in *Supplementary material 1*.

Statistical Analyses

Prior to statistical analyses, the effects of age, body mass index (BMI, kg/m²), medication-use, systolic and diastolic BP were regressed out of the cardiovascular autonomic variables in SPSS version 12.0.2 (SPSS Inc., Chicago, IL, USA), to take these confounders into consideration without losing statistical power. Of the monozygotic twins (N=148), 15 individuals reported medication use (1= Anti-hypertensive, 14 = Other, not cardioactive, medication). Of the dizygotic twins (N=102), 32 individuals used medication (2= Antihypertensives, 30= Other, not cardioactive, medication). Those using cardioactive medications were excluded from the analysis. The effect of medication is found to be marginal (Riese et al., 2006), we therefore decided to account for its effect by regressing this out prior to analyses.

As for blood pressure, this was regressed out due to its known influence on vascular stiffness (including carotid artery stiffness) and can thus influence baroreflex sensitivity (Mukai, Gagnon, Iloputaife, Hamner, & Lipsitz, 2003). We also regressed out the effects of age from the anxiety variables in R statistical environment and subsequently used residuals in the analysis.

Twin Model fitting Analysis

We analysed the relationships between anxiety and the three cardiovascular autonomic function measures in a multivariate twin model. The classical twin design rests on the comparison between monozygotic (MZ) and dizygotic (DZ) twins; MZ twins share 100%, whereas DZ twins share on average 50% of their segregating DNA. We initially estimate similarity in MZ and DZ twin pairs within and across traits (twin correlations). Using biometrical structural equation modelling (SEM), variance of traits are further decomposed into three latent factors; additive genetic influences (A), common/shared environmental influences (C) which contribute to twin pair similarity (e.g. environmental factors affecting both twins in one family) and (iii) non-shared environmental factors (E), that contribute to differences between twins within one pair (including random measurement error).

Through standardisation, the A, C and E factors represent proportion of variance. For example, heritability (a^2) of a trait is the proportion of variance in that trait due to genetic differences in the population. The same principle applies for standardising environmental influences (c^2 and e^2). Covariance between traits are also decomposed into aetiological correlations (denoted r_A, r_C and r_E) which suggest the extent to which the A, C and E factors underlying variance for one trait also affects the other. Using this aetiological information, the phenotypic correlation (rPh) between anxiety and cardiovascular autonomic measures can also be decomposed.

Our multivariate model features a latent anxiety factor (L_{ANX}), ascertained by the twins' self-reported anxiety and co-twin sisters' report (four measures). The latent BRS, HF_{IBI} and mIBI

factors (L_{BRS}, L_{HF}, L_{IBI}), were each determined by four measurements during the experimental conditions. In addition to specific measurement error, we also modelled a 'rater-bias' component for the anxiety variables. This considers the additional covariance between a twin's self-report and what is reported by the co-twin and separates rater bias and unreliability from the latent anxiety factor. The analyses in this paper follow previous procedures using the same sample (Riese et al., 2007) with model-fitting conducted in the OpenMx package in R (Neale, Hunter, Pritikin, et al., 2016; Neale & Miller, 1997). The full model-fitting procedure is further detailed in ***Supplementary material 2***.

Results

Table 1 presents general characteristics of the sample and **Table 2** details means (S.D) for the four experimental conditions.

[Tables 1 & 2 about here]

<u>Phenotypic factor model</u>

The phenotypic factor model (**Figure 1**) obtains correlations between the latent anxiety and the three cardiovascular autonomic factors (-2 Log L= 11457.38 , df= 3542, AIC= 4373.381). Latent anxiety significantly negatively correlated with BRS (r = -0.24, 95% CI = -.42 / -.05). The relationship between latent anxiety and mIBI was also negative but non-significant (r = -.15, 95% CI = -.33 / .03) and the same with HF_{IBI} (r = -.16, 95% CI = -.34 / .03). **Table 3** outlines these phenotypic (rPh) and other intraclass correlations.

[Insert figure 1 here]

[Table 3 about here]

<u>Genetic factor model</u>

The full genetic SEM model, estimating all parameters (-2LL = 11408.13, df= 3510, AIC = 4388.129), was used as a comparison for nested sub-models to determine the model with best fit. The final model fixed the A and C specific effects (on measured variables) to zero, apart from one A specific effect on the state anxiety variable (as it was too substantial to drop from the model). There was no significant reduction in fit between the full and final model, and a lower AIC observed (Δ -2LL (Δdf) = 23.35(31), p = .84). We henceforth report results of the final model (Figure 2).

Standardised variance components (a^2, c^2, and e^2) of each latent factor were estimated (**Table 4**), with heritability (a^2) estimates being moderate and significant for mIBI. Genetic correlations were not significant between latent anxiety and any of the cardiovascular autonomic measures (**Table 5**): with BRS (r_g= -.18, 95% CI = -1, 1), with mIBI (r_g = -.13, 95% CI = -1, 1) or with HF_{IBI} (r_g = -.13, 95% CI = -1, 1). We did, however, find that the phenotypic relationship between anxiety-BRS was mostly explained by shared environmental influences (58%). Rater bias components were non-significant for all the anxiety variables.

[Tables 4 & 5 about here]

[Insert Figure 2 here]

Discussion

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This study investigated the genetic and environmental aetiology and relationships between a latent anxiety and three cardiovascular autonomic function factors; baroreflex sensitivity (BRS), mean inter-beat interval (mIBI) and heart rate variability in the high frequency band (HF$_{IBI}$). We report a significant negative correlation between the latent anxiety and BRS factors, mostly driven by shared environmental influences. We did not obtain significant genetic/ environmental correlations.

Aetiology of Anxiety and Autonomic measures

We report moderate heritability estimates for the latent anxiety and cardiovascular autonomic measures (35-44%) with the estimate for mean inter-beat interval (IBI) being significant. Heritability of the anxiety factor is consistent with previous literature (López-Solà et al., 2014; Nivard et al., 2015). Although the estimates for the autonomic measures are lower than those of a previous study using the same sample, reporting heritability estimates ~50% (Riese et al., 2007), the confidence intervals are wide in both studies indicating the need for larger sample sizes to increase certainty around the point estimates. There are also other possible reasons for this; firstly, our study fits a latent anxiety factor rather than neuroticism, a different psychological construct, which can change the correlation structure of the twin model. Our cross-twin cross-trait correlations (**Table 3**), suggest a higher influence of shared environmental effects explaining covariance between factors, whereas this pattern is reversed in the previous paper (where MZ correlations are higher than DZ, suggesting more genetic influence), although again with wide confidence intervals.

Secondly, we report a different final model compared to the previous paper and have kept the

substantial specific genetic influence on state anxiety as opposed to dropping the parameter completely. Thirdly, our analyses used OpenMx (Neale, Hunter, Pritkin, et al., 2016), a relatively new modelling package with a different optimizer than that used in the previous study. Taken together, replication of this study in a larger sample is required to improve precision in estimating genetic and environmental effects.

Relationships between Anxiety and Cardiovascular Autonomic measures

Higher scores on a latent anxiety factor was correlated with lower BRS, suggesting reduced ability to respond to, and regulate blood pressure with increasing levels of anxiety. Negative correlations were also found between anxiety-IBI and HF_{IBI} but did not reach significance. This may highlight a specific link between anxiety-BRS, but also necessitates further evidence, as this is currently the first twin study combining the three cardiovascular autonomic measures with anxiety. Nevertheless, our results lend support to the neurovisceral integration model, whereby autonomic flexibility may be reduced with elevated levels of anxiety and stress (Friedman, 2007). We did not, however, find evidence for pleiotropic genetic effects. Aside from sample size, our participants are relatively healthy in terms of anxiety symptoms, creating a restricted range of scores, possibly decreasing power further. The relationship could also be largely environmentally driven, as supported by previous work on cardiovascular autonomic functioning with genetic effects being minimal (Osztovits et al., 2011). The phenotypic relationship between anxiety and BRS was mostly accounted for by shared environmental influences. These are environments that make twins similar such as the home environment, school attended and peer groups. These environments may foster an anxious profile for the twins reducing BRS or vice versa. Nevertheless, this should be interpreted with caution, given that the shared environmental correlation is non-significant

and difficulty in pinpointing exact environmental factors from this study alone.

<u>**Strengths**</u>

Our study has several strengths. This is, to our knowledge, the first multivariate twin analysis combining anxiety symptoms (both state and trait anxiety) with all three cardiovascular autonomic measures (mIBI, HF_{IBI}, BRS). Anxiety was investigated in a dimensional, symptom-based context as opposed to a diagnostic perspective, tapping into the anxiety spectrum rather than a relatively restricted range of scores. Secondly, our study offers a behaviour genetic perspective. Although previous work suggests negative associations between anxiety symptoms and measures of autonomic function, they do not employ twin analyses into the phenotypic, genetic and environmental relationships. Although we were likely underpowered to detect such genetic and environmental effects, our design allowed us to also test these parameters. Our finding of a negative association between anxiety and BRS, directly supports previous research and adds to the role of autonomic dysfunction with anxiety phenotypes (Sanchez-Gonzalez et al., 2015; Virtanen et al., 2003; Watkins et al., 2002). Third, we controlled for various confounders, including BMI and medication and as our study was made up of a homogeneous female sample, results were not confounded by sex and a wide age span.

<u>**Limitations and future directions**</u>

Firstly, we are limited by our small female sample. Although eliminating sex and age-specific confounds, replication is required in larger samples for increased statistical power. It is also worth including males, considering sex differences in anxiety and autonomic functions reported at the phenotypic and genetic level (Koenig et al. 2017; McLean and Anderson

2009). Also, both anxiety (Lee, Gatz, Pedersen, & Prescott, 2016) and cardiovascular health (North & Sinclair, 2012; Paneni, Diaz Cañestro, Libby, Lüscher, & Camici, 2017) is influenced by age, prompting further research with different age groups. Furthermore, genetic and environmental influences can be age dependent, such that new age-specific factors may emerge overtime (Franić, Middeldorp, Dolan, Ligthart, & Boomsma, 2010). A longitudinal twin design can best decipher the stability and change in such genetic and environmental influences.

Secondly, we measured autonomic functioning in a laboratory setting, with 5-minute recordings for each condition. While this may not reflect everyday autonomic functioning, experimental tasks are a widely used, accurate method to investigate autonomic performance (Chalmers, Heathers, Abbott, Kemp, & Quintana, 2016; Riese et al., 2006, 2007). Short-term measurements of autonomic functions are found to be highly reliable, especially with healthy adults (Sandercock, Bromley, & Brodie, 2005). Future studies, however, may use the growing work on ambulatory assessment of anxiety and cardiovascular functioning in real-time through mobile/ wearable technology. This can provide data that is both longitudinal and reflective of everyday tasks. Thirdly, as autonomic functions were measured at one time-point in the TWINS study, we were limited by a cross-sectional design. Although causal inference (e.g. between anxiety and BRS) is not possible here, future work may focus on designs that are closer to establishing causality, including longitudinal research and combining causal inference methods with the twin design (Minică, Dolan, Boomsma, de Geus, & Neale, 2018).

Fourth, our findings are based on European participants. Results may differ across non-

western samples, especially considering culture-specific anxiety syndromes (Koydemir & Essau, 2018) and differences in cardiovascular health according to ethnicity (El-Gabalawy, Mackenzie, Pietrzak, & Sareen, 2014; Li et al., 2009). Further cross-cultural research that incorporates genetically sensitive designs may decipher similarities and differences in anxiety and cardiovascular health markers.

Finally, our results are preliminary. Although we find that environmental influences shared between twins mostly explains the overlap between anxiety and lower baroreflex control, genetic influences should not be ruled out. More recently, genome-wide association studies (GWAS), have begun highlighting common genetic variants associated with anxiety (Alves et al., 2017; Gottschalk & Domschke, 2017; Purves et al., 2019) and cardiovascular functions (Nolte et al., 2017; Sigurdsson, Waldron, Bortsov, Smith, & Maixner, 2018). This approach provides insight into genetic aetiology at a molecular level and paves the way for polygenic risk scores to identify individuals at-risk for anxiety and autonomic dysfunction. There is, therefore, scope to expand on the twin design reported here. Future clinical applications may involve screening individuals with anxiety for cardiovascular autonomic dysfunction to identify and prevent future cardiovascular complications.

Conclusion

In conclusion, higher scores on a latent anxiety factor were associated with lower baroreflex sensitivity and shared environmental factors may possibly underlie this. The ability to respond to and regulate blood pressure may therefore be compromised with increasing levels of anxiety symptoms. Higher anxiety was also related to lower inter-beat interval (mIBI) and heart rate variability (HF_{IBI}), but these associations were not significant. We did not find

evidence for pleiotropic effects (i.e. relationships due to shared genetic influences), although

further research with larger sample sizes are required to determine these findings. Our results

suggest a link between anxiety and lower baroreflex control and adds to the literature on the

governing role of autonomic dysfunction in the associations between anxiety and

cardiovascular health.

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Table 1. General characteristics of the twin sample with means (S.D).

	Monozygotic (N=148)	**Dizygotic (N=102)**
Age in years	22.56 (3.76)	22.45 (3.35)
Anxiety measures		
Profile of Mood states (ANX 1) *0 = No/ minimal anxiety;* *1 = Report level of anxiety*	0 = 137/ 148 (93%) 1 = 11/148 (7%)	0 = 93/102 (91%) 1 = 9/102 (9%)
State anxiety (ANX 2) *Range: 20-53*	30.58 (5.74)	32.25 (6.37)
HSCL Anxiety sum score (ANX 3) *Range: 0-15*	2.68 (2.25)	3.06 (2.88)
HSCL Anxiety Sibling score (ANX 4) *Range: 0-15*	2.15 (2.16)	2.10 (2.77)

Note that the Profile of Mood states variable is ordinal in nature, and we have therefore reported

proportions.

Table 2. Means (S.D.) for BRS, mIBI and HF$_{IBI}$ in each of the four experimental conditions.

Cardiovascular autonomic function measure	Experimental condition			
	Rest 1	Stress 1	Stress 2	Rest 2
BRS (ms/mmHg)	9.25 (3.65)	8.56 (3.31)	8.84 (3.58)	8.65 (3.66)
IBI mean (ms)	770.33 (107.77)	755.51 (113.14)	747.78 (109.71)	758.32 (110.73)
HRV - HF$_{IBI}$ (log (ms^2))	6.89 (.90)	6.67 (.83)	6.69 (.84)	6.78 (.89)

BRS, Baroreflex sensitivity; mIBI, mean IBI; HF$_{IBI}$, Heart rate variability with IBI power in the 0.15–

0.40 Hz frequency band.

Table 3. Twin correlations within and across traits (95% CI) for MZ and DZ twins separately.

Latent factors	Within-twin (rPh)	rMZ	rDZ
Within trait		Cross twin – Within trait correlations	
ANX – ANX		**.74 (.45 , .99) ***	**.48 (.13, .76) ***
BRS – BRS		**.54 (.31 , .70) ***	.03 (-.32 , .39)
mIBI – mIBI		**.55 (.36 , .69) ***	.01 (-.32 , .35)
HF$_{IBI}$ – HF$_{IBI}$		**.52 (.30 , .68) ***	.12 (-.23 , .45)
Cross trait		Cross twin - Cross trait correlations	
ANX – BRS	**-.24 (-.42 , -.05) ***	-.17 (-.39 , .04)	**-.28 (-.50 , -.02) ***
ANX – mIBI	-.15 (-.33 , .03)	-.08 (-.29 , .12)	-.23 (-.44 , .02)
ANX – HF$_{IBI}$	-.16 (-.34 , .03)	-.13 (-.34 , .08)	**-.29 (-.50 , -.04) ***
BRS – mIBI	**.65 (.55 , .74) ***	**.35 (.17 , .51) ***	-.09 (-.37 , .22)
BRS – HF$_{IBI}$	**.69 (.59 , .77) ***	**.35 (.16 , .51) ***	.00 (-.30 , .32)
mIBI – HF$_{IBI}$	**.49 (.37 , .60) ***	**.24 (.07 , .40) ***	-.04 (-.31 , .26)

Correlations derived from the Phenotypic Common Pathway model. rPh = phenotypic correlation, i.e.

the within-twin cross-trait correlations; rMZ = Monozygotic twin correlation; rDZ = dizygotic twin

correlation. ANX= Anxiety; BRS= Baroreflex sensitivity mIBI= latent inter-beat-interval factor;

HF_{IBI}, heart rate variability with IBI power in the 0.15–0.40 Hz frequency band. HF_{IBI} values were log transformed. ***** = significant correlation (indicated by the 95% CI not spanning zero).

Table 4. Standardised variance components (95% CI) of latent factors.

Latent factor	a^2	c^2	e^2
ANXIETY	.42 (.00 , .88)	.31 (.00, .72)	**.27 (.09, .54) ***
BRS	.42 (.00 , .66)	.09 (.00, .47)	**.49 (.32, .72) ***
mIBI	**.44 (.07 , .65) ***	.08 (.00, .39)	**.48 (.33, .67) ***
HF_{IBI}	.35 (.00 , .64)	.15 (.00, .50)	**.50 (.34, .71) ***

Contribution of genetic (a^2), common environmental (c^2), and unique environmental (e^2) influences on the variance of the latent anxiety and the three autonomic factors ***** = Significant (indicated by the 95% CI not spanning zero).

Table 5. Genetic and environmental correlations (95% CI) between latent factors.

Latent factors	Genetic correlation (r_g)	Common environmental correlation (r_c)	Unique environmental correlation (r_e)
ANX – BRS	-.18 (-1 , 1)	-.86 (-1 , 1)	-.06 (-.43, .34)
ANX – mIBI	-.13 (-1 , 1)	-.90 (-1 , 1)	-.14 (-.47, .21)
ANX – HF_{IBI}	-.13 (-1 , 1)	-.99 (-1 , 1)	.00 (-.36, .37)
BRS – mIBI	.66 (-1 , 1)	.55 (-1 , 1)	**.67 (.49, .81) ***
BRS – HF_{IBI}	.63 (-1 , 1)	.82 (-1 , 1)	**.73 (.54 , .86) ***
mIBI – HF_{IBI}	.33 (-1 , 1)	.93 (-1 , 1)	**.55 (.34 , .71) ***

* = Significant correlation (indicated by the 95% CI not spanning zero).

Figure 1 - Phenotypic Factor Model (including a rater bias component)
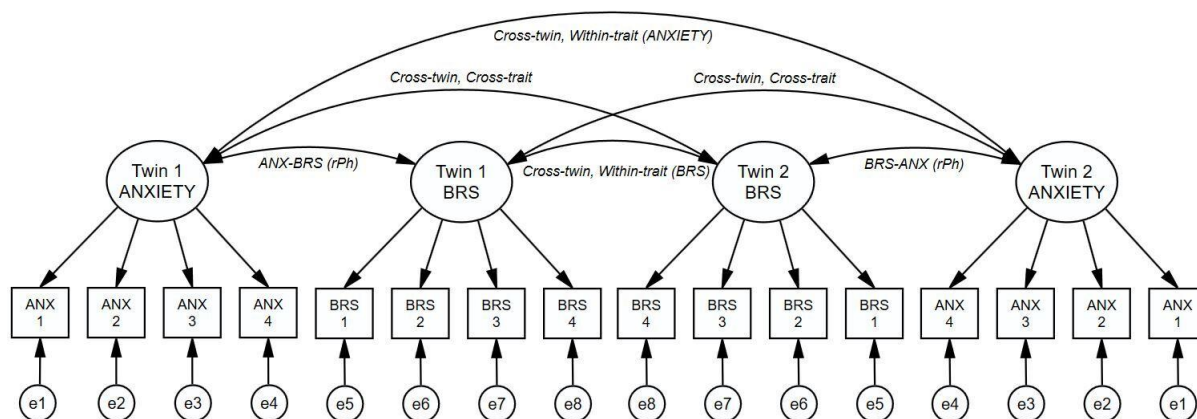
Phenotypic associations between latent anxiety and baroreflex sensitivity (BRS) factors (for a twin pair). For simplicity, HRV (HF_{IBI}) and inter-beat interval (mIBI) were omitted from this figure. Latent (unobserved) factors are depicted in circles, observed (measured) variables shown in rectangles. Twin 1/ 2 ANXIETY = Latent anxiety factor for twin 1/2; Twin 1/2 BRS = Latent baroreflex sensitivity factor for twin 1/2. Anx 1 = Profile of Mood states anxiety; Anx 2 = State anxiety; Anx 3 = Hopkin's Symptom checklist anxiety; Anx 4 = Co-twin sibling report of anxiety via the Hopkins symptom checklist. BRS 1-4 = Four measurements of BRS during the experimental task. Arrows running from latent factors to measured variables indicate path loadings, paths between latent factors represent the phenotypic correlations. ANX-BRS (rPh) = Phenotypic correlation between latent factors. Cross-twin, Within-trait (ANXIETY/BRS) = Correlations across twins, within latent anxiety and BRS factors; Cross-twin, Cross-trait = Correlations across twins and across latent anxiety and BRS factors. e1-e8 = Specific unique environmental effects on the measured variables.

Figure 2 - Genetic factor model (including a rater bias component)
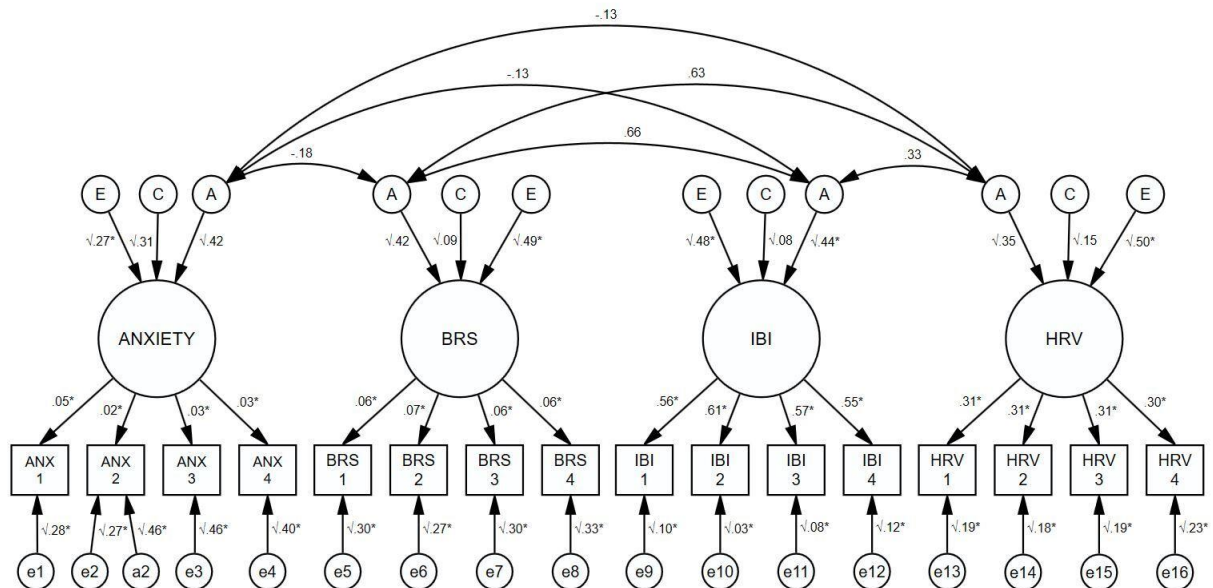
The genetic model depicting genetic and environmental contributions to Anxiety, BRS, IBI and HRV (For an individual). Circles depict latent (unobserved) factors, rectangles are observed (measured) variables. ANXIETY = Latent Anxiety factor; BRS = Latent Baroreflex sensitivity factor; IBI = Latent IBI factor; HRV = Latent Heart rate variability factor. ANX 1 = Profile of Mood States anxiety; ANX 2 = State Anxiety; ANX 3 = Hopkin's Symptom checklist anxiety; ANX 4 = Co-twin sibling report of anxiety via the Hopkins symptom checklist. A = Additive genetic effects; C = Shared environmental effects and E = Unique environmental effects. e1 – e16 = Unique environmental effects specific to observed variables; a2 = Genetic specific effect on state anxiety. Arrows represent path loadings. Paths running between latent A factors represent genetic correlations. For simplicity, C and E correlations as well as the rater bias component are not shown. Table 5 details the full account of aetiological correlations between factors.

Abbreviations

ANX: Anxiety**; BP:** Blood Pressure; **BRS:** Baroreflex Sensitivity; **mIBI:** Inter-beat Interval mean; **HF_{IBI}:** Heart rate variability in the high frequency band, IBI power in 0.15-0.40 Hz, ms²; **HSCL**: Hopkin's Symptom Checklist; **TWINS:** Twin Interdisciplinary Neuroticism

Study

Ethical standards

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

Author Contributions

ZN and FR conceived the study. FR and HR were involved in the TWINS study formulation and data pre-processing. ZN performed the twin modelling analysis supervised by FR. ZN and FR wrote the manuscript. AR provided expertise regarding the cardiovascular autonomic measures. All authors contributed to manuscript revision, read and approved the submitted version.

Acknowledgments

Conflict of Interest Statement

None

Supplementary material

Supplementary material 1: Experiment details & quality control

Supplementary material 2: Twin model fitting analysis using structural equation modelling

(SEM)

Supplementary material for Chapter 3

Supplementary material 1. Experiment details & quality control

Twin pairs typically arrived at 9:00 and the protocol started with blood samples taken as well as weight/height/circumference assessments. Cardiovascular autonomic functions (BRS, IBI, HRV) were assessed in an experimental laboratory task with four standardised conditions in the following order: Rest (R1), Stress with visual feedback (S1), Stress with auditory feedback (S2) and Rest (R2). Stress conditions were employed to measure the shift in sympathetic/parasympathetic balance. The stress tasks exposed participants to a modified version of the 'emotion face dot probe task' (Mogg & Bradley, 1999; Riese et al., 2006a), involving a series of trials whereby a pair of faces was presented for 19 ms, followed by a mask for 50 ms. Following this, dots appeared in the location previously occupied by the two masked faces: 11 dots on one side, and three or four dots on the other side. Participants had to indicate whether three or four dots appeared as quickly as possible using a button response.

The task is modified in that it involves the use of dots for the response frame rather than horizontal or vertical semi-colons. Visual feedback was given by presenting the correct number of dots for 1000 ms in the centre of the screen; "3 stippen! (3 dots!)" or "4 stippen! (4 dots!)", in the Courier New font with 18 font size. Auditory feedback involved exposing participants to 100dB white noise for 500 ms when a wrong response was given. The auditory feedback was presented to participants twice before this second session started. Cardiovascular measurements began after the participants relaxed in a sitting position for a minimum of 10 minutes. Each experimental condition lasted approximately 5 minutes.

Measurements were excluded if signal recording failed. For continuous blood pressure (BP)
and heart rate, artefacts, outliers, and missing values were corrected for using linear
interpolation of four data points surrounding the artefact. Visual inspection led to 976
measurements suitable for BRS calculation in the CARSPAN spectral analysis program
(Mulder, 1988; Robbe et al., 1987). The method has also been the basis of calculating BRS in
various other studies (Althaus et al., 2004; Dietrich et al., 2006; Lefrandt et al., 1999; Van
Roon, Mulder, Althaus, & Mulder, 2004). The program enables discrete Fourier
transformation of non-equidistant systolic BP and IBI series. These time series were
corrected for artefacts and checked for stationarity. BRS was defined as the mean between
spectral IBI variability and BP variability values in the 0.07-0.14 frequency band, expressed
in ms/mmHg. The gain in the 0.07–0.14 Hz frequency band is influenced by both branches of
the autonomic nervous system (Akselrod et al., 1985) and it has been demonstrated that the
narrow band (around 0.10 Hz) is valid for determining changes in short-term blood pressure
regulation (Robbe et al., 1987). For respiration, spectral power values were calculated, which
were used in the BRS quality control procedure (Jorna, 1992).

The quality of the dataset was assured by excluding:
(1) 20 BRS values obtained based on less than three frequency points (i.e. less than 3 out of
the 8 points in the 0.07-0.14 frequency band);
(2) 13 BRS values that were based on measurements that had more than 10% of the BP signal
corrected by CARSPAN and/or contained too many artefacts (that is, time-series with
supraventricular extra systoles, showing signal gaps of more than 5s of IBIs and/or more than
10s in systolic BP signals);

(3) 9 BRS values obtained from measurements lasting less than 100s; and

(4) 19 BRS values based on unreliable IBI spectral power values due to power influences from the respiration signal in the 0.07–0.14 Hz band, caused by slow breathing (during normal breathing the respiration peak can be expected around 0.25 Hz).

Participants with no reliable BRS values were excluded in analyses of IBI and HRV. Two participants' HRV measurements deviated more than 3 S.D. from the mean and were also excluded. Two participants were excluded because of supraventricular extrasystoles (8 BRS values), and 31 BRS values were excluded because of other reasons such as talking, coughing during the measurement, or IBI power in the 0.15– 0.50 Hz band instead of the 0.07–0.14 Hz band (Riese et al. 2006).

Supplementary material 2. Twin model fitting analysis using structural equation modelling (SEM)

The parameter estimates of the full 'ACE' model, those of subsequently fitted reduced models and 95% confidence intervals were estimated using maximum likelihood methods in the OpenMx package in R (Neale et al., 2016; Neale & Miller, 1997). Goodness of fit of models were determined using Akaike's information criterion (AIC; Akaike, 1987) and the χ^2 statistic. The AIC judges the fit of the model (χ^2) relative to the number of parameters; a lower AIC shows goodness of fit and parsimony, indicating whether to accept or reject further sub-models.

Phenotypic factor model

The phenotypic pathway model estimated MZ and DZ twin correlations between the latent factors (**Figure 1**). We applied constraints to this model to obtain: (a) one set of within-twin (within individual), cross-trait correlations between ANX, BRS, HRV and IBI (e.g. ANX Twin 1 –BRS Twin 1). This was regardless of twin order or zygosity group. Additionally, (b) one set of cross-twin cross-trait correlations for MZ and DZ pairs separately (e.g. MZ; ANX Twin1 –BRS Twin 2). This was independent of twin order (e.g. ANX Twin1 – BRS Twin2 = ANX Twin2 – BRS Twin1). Finally, (c) the cross-twin within-trait correlations (e.g. ANX Twin1 – ANX Twin2) were free to vary across zygosity groups.

Genetic factor model

Classical twin models estimate the effects of latent (unobserved) genetic and environmental influences on the variance of an observed trait. The power to estimate these variance components is through the differences in covariance (or correlation) of the trait among MZ and DZ twin pairs. The cross twin within-trait correlations allow variances of each latent factor to be decomposed into additive genetic (A or a^2), common environmental (C or c^2) and unique environmental effects (E or e^2). The power to distinguish between different sources of covariance comes through the cross-twin cross-trait correlations (e.g. ANX Twin1 –BRS Twin 2). If the phenotypic relationships between latent anxiety and autonomic factors are significant, this would imply common aetiology and significant cross-twin cross-trait correlations suggest that this aetiology is familial. The ratio of the MZ/DZ cross-twin cross-trait correlations indicate to what extent the common aetiology is genetic or environmental in origin; a 2:1 ratio suggests the effects of A, a 1:1 ratio suggests the effects of C and nonsignificant cross- trait cross-twin correlations suggest that the common aetiology is due to E (Neale & Cardon, 2013).

Correlations between latent factors were modelled as a function of their latent A, C and E influences. The model is expressed in correlated factors: six each for the A, C and E factors (**Figure 2**; only genetic correlations, r_g, are shown). The degree to which latent A factors contribute to the phenotypic correlation between any two latent factors is gained by multiplying square roots of the standardized estimates (a^2) of the latent phenotypes with their matching genetic correlation (r_g). The same procedure is done to obtain the contributions of C and E to the phenotypic correlation. The total phenotypic correlation is therefore a sum of the A, C and E correlations.

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Summary of chapter 3

This chapter investigated the phenotypic, genetic, and environmental associations between anxiety symptoms and three measures of cardiovascular autonomic functioning. Findings indicate a significant phenotypic association between anxiety and baroreflex sensitivity, suggesting that as anxiety symptoms increase, the ability to regulate blood pressure may decrease (and vice versa). There were no aetiological correlations, except that shared environmental influences were likely to underlie this anxiety-baroreflex sensitivity relationship. In the next chapter, the mental-physical health relationship is explored further, between anxiety and health related quality of life. Given that chapter 3 focused on a western, female only population, the next chapter offers a unique angle bringing this work to a non-western, Sri Lankan population also allowing sex differences to be tested.

Chapter 4 . Associations Between Anxiety Symptoms and Health-Related Quality of Life: A Population-Based Twin Study in Sri Lanka

This chapter is an exact copy of a peer-reviewed publication:

Nas, Z., Zavos, H.M.S., Sumathipala, A. et al. Associations Between Anxiety Symptoms and Health-Related Quality of Life: A Population-Based Twin Study in Sri Lanka. Behav Genet (2021).

Supplementary material is provided at the end of the chapter

ORIGINAL RESEARCH

Associations Between Anxiety Symptoms and Health-Related Quality of Life: A Population-Based Twin Study in Sri Lanka

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Abstract

Anxiety not only concerns mental wellbeing but also negatively impacts other areas of health. Yet, there is limited research on (a) the genetic and environmental aetiology of such relationships; (b) sex differences in aetiology and (c) non-European samples. In this study, we investigated the genetic and environmental variation and covariation of anxiety symptoms and eight components of health-related quality of life (QoL), as measured by the short form health survey (SF-36), using genetic twin model fitting analysis. Data was drawn from the Colombo Twin and Singleton Study (COTASS), a population-based sample in Sri Lanka with data on twins (N = 2921) and singletons (N = 1027). Individual differences in anxiety and QoL traits showed more shared environmental (family) effects in women. Men did not show familial effects. Anxiety negatively correlated with all eight components of QoL, mostly driven by overlapping unique (individual-specific) environmental effects in both sexes and overlapping shared environmental effects in women. This is the first study in a South Asian population supporting the association between poor mental health and reduced QoL, highlighting the value of integrated healthcare services. Associations were largely environmental, on both individual and family levels, which could be informative for therapy and intervention.

Keywords Anxiety · Quality of life · Twin study · Sex differences · Non-western samples

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Introduction

Anxiety symptoms are highly prevalent in the population (Mehta et al. 2003; Mallorquí-Bagué et al. 2016), and can be viewed as a continuum with healthy individuals on one end and those with anxiety disorders on the other. Anxiety symptoms impact not only emotional wellbeing, but are also associated with chronic health problems (Davies and Allgulander 2013; El-Gabalawy et al. 2014; Tang et al. 2017), bodily pain (Lerman et al. 2015), fatigue (Vassend et al. 2018) and sedentary lifestyles (Bélair et al. 2018; Stubbs et al. 2017; Vancampfort et al. 2018). Social skills and engagement in group activities are also reduced especially with a socially anxious profile (Scharfstein et al. 2011). Together, these limitations substantially impair quality of life (QoL). Yet, these overlaps are often undetected in healthcare settings, with physical health often taking priority. Understanding how anxiety symptoms are related to QoL carries importance for informing healthcare planning and prioritising both mental and physical wellbeing.

Anxiety symptoms, as measured by the GAD-7 (Generalised anxiety), STPI (State anxiety) and APQ (Anxious personality questionnaire), are heritable, with 20–70% of individual differences in symptoms attributable to genetic differences between people in the population (López-Solà et al. 2014; Petkus et al. 2016; Malanchini et al. 2017). Importantly, heritability of anxiety and the relative influence of the environment shows developmental changes, such as genetic innovation arising later on in life (Lee et al. 2016; Petkus et al. 2016). Health-related QoL measures including physical activity (Carlsson et al. 2013; den Hoed et al. 2013), social functioning (McGue and Christensen 2007), fatigue and pain (Vassend et al. 2018) have also been the focus of twin studies. There is, however, limited twin research combining these different areas of health related QoL. Tapping into eight general domains, the short-form health survey (SF-36) is a valid and reliable assessment of health-related QoL. Yet, behaviour genetic research on this measure is sparse. An early twin study on male twins from the US suggests that 17–33% of the variance in the eight domains of the survey can be explained by genetic factors (Romeis et al. 2005). Environment shared by twins had small to negligible effects whereas unique environmental influences explained a large proportion of variance. A later study on Danish twins used a shortened version of the survey, yielding similar heritability estimates ranging 11–35% with most of the variance accounted for by unique environmental effects (Steenstrup et al. 2013). Yet, to our knowledge, no twin studies have combined anxiety with these QoL domains in a genetically informative sample.

There is little information on whether anxiety and health related QoL are correlated due to overlapping genetic or environmental factors i.e. share the same aetiological origin. It is also unknown whether there are sex differences in the aetiological overlap. Previous studies indicate females as disproportionally affected by anxiety symptoms with higher heritability estimates compared to males (Ask et al. 2014). Findings, however, are inconclusive. Studies report small to negligible sex differences in anxiety prevalence and its variance decomposition, again dependent on developmental time points (Lamb et al. 2010; Franić et al. 2010; Durbeej et al. 2019).

Another major limitation of previous work is that most of the evidence comes from western samples, and findings may not necessarily extrapolate across cultures. Twin studies conducted in non-western populations reveal differences in genetic and environmental influences. One such study conducted on Chinese twins (N = 712) finds a modest heritability (23%) for anxiety symptoms in late childhood, decreasing to a negligible effect at mid-adolescence (Zheng et al. 2016). Contrary to twin studies in the western world, shared environmental influences were found to increase substantially overtime. Another study conducted on 620 Chinese adolescent twin pairs yields a much lower estimate for the heritability of anxiety symptoms (9.9%) (Unger et al. 2011). No sex differences were observed in the two studies described. Yet, another study in Chinese children and adolescents (N = 1400) reports not only higher heritability (ranging 50% for self-report and 63% for parent reported anxiety), but also sex differences, whereby heritability of anxiety was higher in girls for self-reported data, but higher in boys for parent-reported data (Chen et al. 2015a). Modest- high heritability estimates (26–48%) were also obtained for studies using large Korean twin samples (Sung et al. 2011; Song et al. 2017, 2019). Despite these studies, research is still behind on the inclusion of South-Asian participants, especially at older age ranges.

The present study uses a South Asian population-based adult twin and singleton sample to investigate (i) the genetic and environmental variance components of anxiety symptoms and health related QoL; (ii) their phenotypic relationships; (iii) the extent to which overlapping genetic and environmental factors underlie their associations and (iv) sex differences in these parameters.

Methods
Sample

We used a representative population sample from the Colombo Twin and Singleton Study (COTASS), as part of the Sri Lankan twin registry (Sumathipala et al. 2013). This is a two-wave cohort study of twins and singletons residing in the Colombo district, a mix of urban and rural environments and home to ~ 2.3 million people. Both mental and physical health was assessed, with the first wave completed in 2005–2007 (Siribaddana et al. 2008) and the second phase (COTASS-2), acquired between 2012 and 2015 (Jayaweera et al. 2018). For this study, we used data from COTASS-2, with twins (N = 2921) and singletons (N = 1027) followed up from the original COTASS study through invitation letters and by telephone. Data collection was conducted during home visits. The sample consisted of a total of 3948 individuals (1676 males; 42.5% and 2272 females; 57.5%) (Table 1). Singletons were significantly older than the twin sample with a mean age of 51.46 and 39.88, respectively (t (1645.2) = − 22.17, p < 0.001).

All participants provided written informed consent. Individuals that did not understand the consent process or the questionnaires due to language barriers were excluded. Participants that had successfully completed one or more study parts were offered 750 LKR (approximately £3.50 GBP) to compensate for their time. COTASS received ethical approval from the Psychiatry, Nursing & Midwifery Research Ethics subcommittee, King's College London, UK

Table 1. Number of individuals included in the analyses, by sex and zygosity group

Zygosity	Males	Females	Total number of individuals
MZ			
Number of individual twins in full pairs[a]	478	668	1263
Number of single twins	55	62	
DZ			
Number of individual twins in full pairs[a]	302	410	850
Number of single twins	63	75	
DZOS			
Number of individual twins in full pairs[a]	343	343	808
Number of single twins	47	75	
Singletons	388	639	1027
Total	1676	2272	3948

MZ monozygotic twins, *DZ* dizygotic twins, *DZOS* dizygotic opposite sex twins

[a]These are individuals who are part of a complete twin pair

(reference number: PNM/10/11-124) and the ethical review committee at the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (reference number: 596/11).

Measures

Anxiety

Anxiety was measured using the *GAD-7* (Spitzer et al. 2006), which captures the presence of generalised anxiety, as indicated in the DSM, over the past 2 weeks. Participants indicated for 7 items how often they were bothered by problems such as 'feeling nervous, anxious or on edge' and 'trouble relaxing', ranging from 0 (Not at all) to 3 (nearly every day). A total anxiety score was derived, ranging from 0 to 21. Raw scores of 0–4 indicate minimal anxiety, 5–9 mild, 10–14 moderate and 15–21 indicating severe anxiety (Spitzer et al. 2006). The GAD-7 has been shown to have excellent psychometric properties, capturing anxiety symptoms in a reliable and valid way (Spitzer et al. 2006; Löwe et al. 2008; Hinz et al. 2017). We also yielded good internal consistency for the GAD-7 measure (Cronbach's α = 0.87).

Health-related quality of life

The *Short Form Health Survey (SF-36)* was used to gauge health related quality of life (Ware and Sherbourne 1992). The 36-item scale measures eight domains of health: general health perceptions (five items); limitations of physical activities due to health problems (physical functioning; 10 items); limitations in usual activities due to physical health problems (role physical; four items); bodily pain (two items); vitality (energy/fatigue; four items); limitations in social activities due to health problems (social functioning; two items); mental health (emotional well-being; five

items) and limitations in usual activities due to emotional problems (role emotional; three items). A final item, named self-reported health transition, is answered by the participant but is not included in the scoring process. Some items on the questionnaire are recoded so that the scores range from 0 to 100, with 100 representing the best state of health, and 0 indicating worst. After recoding, an average score is obtained from the number of items per domain. The SF-36 survey has been widely used, with demonstration of good reliability and validity (Mchorney et al. 1993). A good internal consistency was found, as averaged across the eight domains (Cronbach's α = 0.82).

Analyses

The classical twin design rests on the known genetic difference across monozygotic (MZ; identical) and dizygotic (DZ; non-identical) twins. MZ twins share 100% of their genes, whereas DZ twins share, on average, 50% of their segregating genes. MZ and DZ twins are assumed to have similar shared environments (e.g. in-utero experiences and parental upbringing) and so differences in similarity are attributed to their genetic differences. This information is used in biometrical structural equation modelling (SEM) to disentangle the variance of a trait into three latent influences: additive genetic (A), common environmental (C) contributing to similarity within twin pairs, and unique environmental factors (E), contributing to differences within twin pairs (including measurement error). This model can be extended to bivariate analyses, which further decomposes the covariance between two traits into A, C and E contributions. These aetiological correlations (denoted rA, rC and rE) indicate how much the A, C and E factors underlying individual differences in one trait also affect the other (Rijsdijk and Sham 2002). These correlations and the standardized variance components are

then used to determine the extent to which the phenotypic correlation (rPh) between anxiety symptoms and each of the QoL scales is due to correlated A, C and E factors (rPh-A, rPh-C and rPh-E, respectively).

Furthermore, twin models can test for sex differences in the aetiology of traits and the aetiological overlap between traits. Including same-sex and opposite-sex twin pairs allows testing for (a) qualitative sex differences—different genetic and environmental factors influencing variance and covariance of traits across sex and (b) quantitative sex differences—whereby the same genetic and environmental factors influence variance/covariance but differ in magnitude across male and female twin pairs. We began with a full bivariate sex limitation model testing for both qualitative sex differences (first for A then for C) and quantitative sex differences in the variance and covariance of anxiety and QoL variables, allowing all parameters to vary across sex (full heterogeneity model). We follow this by testing for quantitative sex differences only. A non-significant decline in fit between the model allowing quantitative sex differences only indicates that there are no qualitative sex differences. This is followed by a nested homogeneity model which equates all A, C and E path estimates across males and females. To detect the best-fitting model, differences in minus twice log likelihood (-2LL) (distributed as χ^2) were examined between nested models, in addition to the Akaike's Information Criterion (AIC) whereby a lower AIC generally indicates a better fit (Rijsdijk and Sham 2002; Neale and Cardon 2013). We used scores on the anxiety and QoL scales as continuous variables, regressed by age and sex and log transformed to mini-mise skew. The only exception to this was social functioning, where a threshold liability model was fitted to a dichotomous variable in a combined ordinal-continuous analysis with anxiety symptoms. Twin model fitting was conducted using the OpenMx statistical package in R (Neale et al. 2016).

Singletons were also included in analyses. Although they cannot contribute to information on the A, C and E variance and covariance decomposition of the genetic model, they add information on the phenotypic variances and covariances of variables and are therefore included in the genetic analyses, just like incomplete singleton twins.

Prior to fitting genetic models, we ran a fully saturated model for each variable followed by a sub model in which variances were tested for equality across sex (Supplementary Table V, Sub 1 models). For seven of the nine variables, this constraint resulted in a significant reduction in fit. In the univariate genetic analyses, we therefore proceeded to testing scalar sex-limitation models, whereby the same aetiology is specified across sex but allowing differences in variances. For most variables, however, this scalar model was a poor fit in comparison to the quantitative heterogeneity model, except for physical functioning, emotional wellbeing, and pain. In the bivariate genetic analyses, we therefore

fitted a hybrid model for anxiety and these scales, specifying male and female ACE components for anxiety and a scalar variance inequality ACE model for the scale variables. In addition, for the bivariate analysis of anxiety and the energy/fatigue scale, we used a hybrid model specifying a homogeneity model for this scale, as the univariate analyses indicated that a homogeneity model does not result in a significant reduction in fit. Bivariate model fit statistics are detailed in Supplementary Table VI.

We also conducted post-hoc MZ twin differences analyses (Pike et al. 1996), to investigate the unique environmental component further. As MZ twins do not differ in their genetic makeup and shared environment, any differences observed is an index of their unique environmental experiences. The MZ-difference design focuses on relative difference scores within twin pairs (twin 1 -twin 2). Here, we calculated difference scores on a number of stressful life events, as measured via the 56-item life-threatening experiences questionnaire culturally adapted for the Sri-Lankan population (Brugha and Cragg 1990). These difference scores are then correlated with relative difference scores on outcome measures (here being GAD-7 anxiety symptoms and the QOL scales). If MZ twins who experienced more stressful life events also showed higher levels of anxiety and lower levels of QOL than the co-twin who experienced less stressful life events, then we can infer that stressful life events might be components of unique environmental variances and covariances of anxiety and QOL.

Results
Descriptive statistics

Descriptive statistics on age, anxiety symptoms and QoL symptoms for each study group is detailed in Table 2. The majority of individuals had minimal or no anxiety, with 8.5% mild, 2.3% moderate and 1.6% with severe anxiety symptoms according to cut-offs provided by Spitzer et al (2006). The distribution of anxiety scores is given in Supplementary Fig. 1. Females reported significantly higher anxiety symptoms than males, with a mean score of 1.54 and 2.10 respectively (t (3852.3) = − 5.4, p < 0.001). Females also report lower health related QoL in comparison to males for all eight scales of the SF-36. Details of these formal tests can be found in Supplementary Table I.

Singletons had significantly higher self-reported anxiety symptoms compared to twins, with a mean score of 1.76 and 2.16 respectively (t (1604) = − 3.18, p = 0.001). This effect remained even after accounting for age (t (1604.2) = − 3.55, p < 0.001). Twins and singletons also showed significant differences on four out of the eight health related QoL scales; general health (t (1712.9) = 5.09, p < 0.001), physical

Table 2. Means (SD) of Age, Anxiety symptoms & health related quality of life (QoL) measures

	MZM	DZM	MZF	DZF	DZOS	Singleton males	Singleton females
Age	37.53 (12.49)	39.41 (13.02)	39.21 (12.83)	43.09 (14.07)	40.28 (13.19)	52.48 (15.45)	50.84 (14.32)
Anxiety	1.53 (2.75)	1.29 (2.62)	1.90 (3.06)	2.01 (3.78)	1.83 (3.30)	1.85 (3.48)	2.36 (3.75)
General health	63.26 (14.97)	62.83 (14.39)	61.45 (16.35)	60.28 (16.94)	61.01 (15.88)	60.94 (15.04)	57.14 (17.71)
Physical functioning	93.82 (14.90)	93.95 (13.36)	88.53 (19.25)	88.05 (19.24)	91.39 (17.06)	87.12 (22.62)	82.94 (21.53)
Role of physical problems	86.11 (31.72)	88.67 (29.47)	81.97 (35.3)	79.24 (37.01)	82.21 (35.43)	79.86 (38.02)	78.21 (39.24)
Emotional wellbeing	78.85 (15.70)	80.5 (14.00)	77.36 (15.58)	76.83 (16.78)	78.57 (15.94)	80.44 (14.15)	76.25 (17.42)
Role of emotional problems	88.7 (28.88)	91.53 (25.10)	85.24 (31.9)	85.91 (31.89)	87.39 (30.65)	88.17 (30.31)	84.56 (34.44)
Energy/fatigue	74.3 (17.15)	75.3 (14.97)	74.56 (16.49)	73.80 (17.07)	74.21 (16.83)	71.16 (16.83)	69.63 (17.68)
Pain	88.7 (19.57)	90.3 (16.99)	85.46 (20.78)	83.56 (21.29)	86.34 (19.52)	87.64 (20.46)	86.50 (21.10)
Social functioning	89.93 (17.81)	91.31 (17.76)	88.5 (19.05)	88.31 (20.86)	89.65 (18.56)	89.22 (21.47)	89.00 (20.48)

The range of the anxiety scale = 0–21; The range of the total SF-36 sub-scales = 1–100

MZM monozygotic male twins, *MZF* monozygotic female twins, *DZM* dizygotic male twins, *DZF* dizygotic female twins, *DZOS* dizygotic opposite sex twins

functioning (t (1503.8) = 8.36, p < 0.001), role limitations due to physical health problems (t (1632.4) = 3.17, p = 0.002) and energy/fatigue (t (1737.5) = 6.70, p < 0.001). The latter scale showed significant differences even after accounting for age (t (1733.3) = 4.29, p < 0.001).

Phenotypic model fitting

We conducted phenotypic analyses for each variable. The cross-twin within-trait correlations (Table 3) suggested little heritability of anxiety across sex, with the MZ:DZ ratio roughly 1:1. The correlations also indicate the influence of shared environmental effects especially for females, since their correlations are significant. As with anxiety symptoms, the phenotypic correlations suggest little or no heritability for the QoL scales with MZ:DZ ratios roughly being 1:1, indicating the effects of shared environment as the familial factor contributing to the variance (individual differences) of the traits. Cross-twin cross-trait correlations can be found in Supplementary Table II and indicate (by the largely non-significant cross-twin cross-trait correlations), for males, unique-environmental sources of covariance

Table 3. Twin correlations (cross-twin within trait) (with 95% CIs)

QoL variable	MZM	DZM	MZF	DZF	DZOS
Anxiety	.09 (− .07/.25)	.08 (− .12/.26)	**.23** **(.12/.33)**	**.30** **(.17/.40)**	.01 (− .08/.11)
General health	**.25** **(.12/.37)**	**.27** **(.10/.41)**	**.31** **(.21/.40)**	**.34** **(.22/.45)**	.11 (.00/.22)
Physical functioning	.12 (− .13/.32)	**.47** **(.23/.61)**	**.38** **(.28/.47)**	**.26** **(.12/.38)**	**.22** **(.10/.33)**
Role of physical problems	.05 (− .11/.20)	.20 (− .01/.37)	**.26** **(.14/.36)**	**.30** **(.17/.41)**	.02 (− .08/.12)
Emotional wellbeing	**.23** **(.12/.34)**	.18 (− .01/.35)	**.31** **(.20/.41)**	**.23** **(.10/.34)**	.10 (.00/.21)
Role of emotional problems	**.19** **(.05/.31)**	.09 (− .14/.30)	**.19** **(.09/.30)**	**.32** **(.19/.44)**	.05 (− .05/.16)
Energy/fatigue	**.18** **(.05/.30)**	**.20** **(.01/.37)**	**.27** **(.16/.37)**	**.28** **(.15/.40)**	**.19** **(.09/.29)**
Pain	.11 (− .02/.24)	**.36** **(.19/.50)**	**.17** **(.06/.27)**	**.24** **(.12/.35)**	.05 (− .07/.16)
Social functioning	**.42** **(.22/.60)**	**.43** **(.16/.65)**	**.43** **(.27/.57)**	**.49** **(.28/.66)**	**.32** **(.15/.47)**

Significant twin correlations are given in bold (as indicated by 95% CI not crossing zero). Please note that cross-twin cross trait correlations can be found in Supplementary Table 2

MZM monozygotic male twins, *MZF* monozygotic female twins, *DZM* dizygotic male twins, *DZF* dizygotic female twins, *DZOS* dizygotic opposite sex twins

between anxiety and QoL measures. For females, however, these correlations are all significant and roughly equal across MZ and DZ pairs, suggesting a shared-environmental (e.g. family environment) source of covariance between anxiety and QoL. The within-individual cross-trait correlations (rPh) were significant and negative between anxiety and all eight components of the health survey, ranging from -0.17 for physical functioning to -0.58 for emotional wellbeing (Supplementary Table III). The actual aetiological components of these results are estimated in the univariate and bivariate genetic models (below).

Univariate model fitting

Table 4 details the standardized variance components of all

variables. For anxiety symptoms, there was little indication

of heritability across sex. In females, a significant proportion of variance in anxiety was explained by shared environment (25%). Unique environmental influences explained a large proportion of variance in anxiety symptoms for males

(91%) and females (75%). This was also the case for the SF-36 scales, with a large proportion of variance explained by unique environmental influences across sex (68–93%). We found significant genetic influence (heritability) for emotional wellbeing (23%), which fit a scalar model so equated across sex. General health, role of emotional problems and social functioning all showed significant amount of shared environmental influences in females (23–28%). The energy/fatigue scale also showed a significant influence of shared environment (22%), which was equal across sex due to a

homogeneity model fitting best.

Bivariate genetic model fitting

Full sex limitation models were fit to the data, specifying both qualitative and quantitative sex differences (first for A then for C; heterogeneity models), which allows parameters to be estimated separately across males and females. Supplementary Table VI details these model fit comparisons. Overall, there were no significant differences between the models specifying quantitative sex differences only and models allowing for qualitative sex differences. The exception to this was the analyses between anxiety-pain, in which there was some evidence for qualitative sex differences in C, though with less reliable standard errors. The C correlations obtained from this qualitative C model were non-significant, detailed in Supplementary Table VII. Homogeneity submodels, whereby path estimates for A, C and E are equated across males and females, were therefore compared to the quantitative heterogeneity models to examine whether the magnitudes of A, C and E effects on anxiety and QoL components differ across sex. A significant decline in fit indicates sex differences. All eight bivariate genetic

Table 4. Standardised variance components of Anxiety symptoms and health related quality of life (QoL) measures in males and females (with 95% CIs) obtained from univariate analyses

QoL variable	Sex	Aetiology (95% CI)		
		A (h²)	**C (c²)**	**E (e²)**
Anxiety	M	.09	.00	**.91**
		(.00/.24)	(.00/.09)	**(.76/1)**
	F	.00	**.25**	**.75**
		(.00/.21)	**(.06/.33)**	**(.66/.83)**
General health	M	.17	.10	**.73**
		(.00/.36)	(.00/.29)	**(.62/.85)**
	F	.04	**.28**	**.68**
		(.00/.30)	**(.05/.39)**	**(.59/.76)**
Physical functioning (scalar model)	M | F	.16	.16	**.68**
		(.00/.39)	(.00/.33)	**(.60/.77)**
Role of physical problems	M	.09	.00	**.91**
		(.00/.23)	(.00/.15)	**(.77/1)**
	F	.00	.27	**.73**
		(.00/.31)	(.00/.35)	**(.64/.82)**
Emotional wellbeing (scalar model)	M | F	**.23**	.04	**.73**
		(.02/.34)	(.00/.21)	**(.66/.81)**
Role of emotional problems	M	.17	.02	**.81**
		(.00/.30)	(.00/.16)	**(.69/.95)**
	F	.00	**.23**	**.77**
		(.00/.16)	**(.08/.31)**	**(.69/.85)**
Energy/fatigue (scalar model)	M | F	.01	**.22**	**.77**
		(.00/.26)	**(.05/.28)**	**(.70/.83)**
Pain (scalar model)	M | F	.00	.15	**.85**
		(.00/.19)	(.00/.21)	**(.77/.90)**
		(.00/.23)	(.00/.00)	**(.76/.99)**
	F	.02	**.23**	**.75**
		(.00/.18)	**(.09/.33)**	**(.67/.84)**

Significant parameters are given in bold (as indicated by 95% CI not crossing zero). These estimates are obtained from the univariate heterogeneity sex limitation analysis. Note that for three variables (physical functioning, emotional wellbeing, and pain) we fit models which specify same aetiology across sex, but allowing different variances. Also note that for the energy/fatigue variable, the homogeneity model fit best, meaning that the aetiology was equal across sex

M males, *F* females, *A* additive genetic influences, *C* common environmental influences, *E* unique environmental influences

homogeneity models resulted in a highly significant reduction in fit. This suggests that the magnitude of genetic and environmental factors influencing anxiety and health related QoL measures were quantitatively different across sex.

Decomposing covariances

Sex differences are evident in the phenotypic correlation breakdown (Table 5). The table decomposes the negative phenotypic correlations into parts due to correlated A (rPh-A), C (rPh-C) and E (rPh-E) factors. In females, there was a large contribution of shared and unique environmental

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Table 5. Phenotypic correlations between anxiety symptoms and health related quality of life (QoL) measures with their corresponding A, C and E components (with 95% CIs) in males and females

QoL variable	Sex	Phenotypic correlation (rPh) (95% CI)	rPh components (95% CI)		
			rPh-A	rPh-C	rPh-E
General health	M	**−.29** (−.33/−.24)	−.01 (−.11/.09)	.00 (−.05/.03)	**−.28** (−.38/−.18)
	F	**−.26** (−.30/−.22)	−.02 (−.10/.08)	**−.12** (−.22/−.04)	**−.12** (−.18/−.06)
Physical functioning	M	**−.22** (−.27/−.17)	−.11 (−.22/.05)	.00 (−.13/.04)	**−.11** (−.21/−.01)
	F	**−.17** (−.21/−.13)	−.02 (−.18/.10)	−.08 (−.16/.06)	**−.07** (−.14/−.01)
Role of physical problems	M	**−.27** (−.31/−.22)	−.05 (−.15/.05)	.00 (−.03/.04)	**−.22** (−.33/.11)
	F	**−.26** (−.30/−.22)	.00 (−.08/.10)	**−.13** (−.23/−.05)	**−.13** (−.19/−.07)
Emotional wellbeing	M	**−.52** (−.56/−.49)	−.05 (−.13/.01)	−.04 (−.11/.01)	**−.43** (−.50/−.35)
	F	**−.58** (−.60/−.55)	−.07 (−.14/.00)	**−.14** (−.20/−.06)	**−.37** (−.43/−.31)
Role of emotional problems	M	**−.43** (−.47/−.39)	−.10 (−.20/.02)	.00 (−.08/.02)	**−.33** (−.45/−.22)
	F	**−.46** (−.49/−.42)	.00 (−.12/.04)	**−.22** (−.29/−.11)	**−.24** (−.30/−.18)
Energy/fatigue	M	**−.40** (−.44/−.37)	−.08 (−.20/.03)	−.03 (−.14/.04)	**−.29** (−.36/−.23)
	F	**−.44** (−.47/−.40)	.02 (−.16/.06)	**−.19** (−.24/−.03)	**−.27** (−.33/−.21)
Pain	M	**−.31** (−.35/−.26)	−.03 (−.13/.06)	−.04 (−.11/.02)	**−.24** (−.33/−.16)
	F	**−.29** (−.33/−.25)	.01 (−.15/.07)	−.13 (−.19/.00)	**−.15** (−.23/−.08)
Social functioning	M	**−.43** (−.48/−.38)	−.15 (−.26/.07)	−.01 (−.25/.02)	**−.27** (−.39/−.16)
	F	**−.44** (−.49/−.40)	.06 (−.33/.06)	−.18 (−.30/.06)	**−.20** (−.29/−.12)

Significant parameters given in bold, as indicated by 95% CI not crossing zero

M males, *F* females, *rPh* phenotypic correlation, *rPh-A* additive genetic component of rPh, *rPh-C* common environmental component of rPh, *rPh-E* Unique environmental component of rPh

effects, whereas in males the main contributor was unique environmental influences.

We found significant shared environmental correlations (rC) in females in four out of the eight analyses, indicating that common environmental factors (e.g. family environment) that contributed to higher scores on anxiety also contributed to lower scores on the QoL variables. Unique environmental correlations (rE) were all significant and negative in males and females. Our bivariate analyses did not yield any significant genetic correlations (rA) between anxiety and any of the QoL components. Estimates of aetiological correlations of the bivariate models (rA, rC and rE) can be found in Supplementary Table IV. Full bivariate model fit statistics can be found in Supplementary Table VI.

Discussion

This is the first twin study examining associations between anxiety symptoms and health related quality of life (QoL) in a South-Asian population. Our study adds to the limited literature surrounding their genetic and environmental aetiology, aetiological correlations, and sex differences. Females reported higher levels of anxiety symptoms and lower self-reported QoL, consistent with research in western populations (Ask et al. 2014; Garratt and Stavem 2017).

Aetiology

Unique (individual-specific) environmental influences explained the majority of variance in anxiety symptoms in males and females and across the health related QoL

measures (68–93%). The large contribution of unique environmental effects (including measurement error) is in line with previous work conducted in western samples (Romeis et al. 2005; Steenstrup et al. 2013). There was no significant heritability for anxiety in males or females, making it a stark contrast to estimates coming from western populations (Trzaskowski et al. 2012; López-Solà et al. 2014; Malanchini et al. 2017). This is also in contrast with data from other Asian samples, including Chinese (Chen et al. 2015a) and Korean samples (Sung et al. 2011). This study, however, is conducted in a South Asian sample, and results should be interpreted in this socio-cultural context.

Overall, genetic factors explained 0–23% of variance in health related QoL. Out of the QoL measures, only emotional wellbeing showed significant heritability. This is comparable to a previous study on male twins from the US (Romeis et al. 2005), and point estimates are similar to a Danish twin sample although using a shortened version of the health survey (Steenstrup et al. 2013). More genetically informative research is required, both in western and non-western samples, to confirm these findings.

The most prominent sex difference is the significant shared environmental influences on females' anxiety symptoms, consistent with data coming from Chinese samples (Chen et al. 2015a, 2016). Several QoL measures in females also show significant shared environmental influence, implying female-specific, common socio-cultural factors under- lying individual differences in these traits. The low heritability estimates are remarkable and could reflect a more variable environment in Asian cultures impacting on mental well-being, particularly for men. Collectivist social norms may also contribute to attenuated heritability estimates, as observed with other phenotypes (Chen et al. 2015b). Environmental trauma is also worth noting, seeing as Sri Lanka was affected by a prolonged civil war (Ball et al. 2009) and a Tsunami in 2004. Our findings therefore reinforce the notion that genetic and environmental influence can be attenuated or amplified across cultural context and environmental variability.

Phenotypic and aetiological correlations

Anxiety was significantly negatively correlated with all eight components of health related QoL. Correlation estimates were similar across sex, and the most important factor explaining these correlations are environmental effects unique to an individual (including measurement error). However, apart from this source of covariance, in females, we find evidence for influence of overlapping shared (family) environmental effects. We did not find evidence for overlap- ping genetic factors in these phenotypic correlations.

We found significant shared-environmental correlations in females, with most being negative, i.e. indicating family

environmental influences that may increase anxiety symptoms and decrease health related QoL. Genetic correlations between anxiety and QoL measures were not significant, indicating that there is not likely to be a common genetic liability or genetic pleiotropic effects. All unique environmental correlations were significant and negative. Hence, the same environment exclusive to an individual can increase anxiety, and also decrease perceived QoL. As the role of the unique environment was so substantial, we decided to investigate this further, conducting post-hoc MZ twin differences analysis (Pike et al. 1996). Briefly, the method is used to isolate unique environmental influences by using relative difference scores for an environmental measure (e.g. stressful life events) and correlate this with difference scores on an outcome variable (e.g. anxiety). We found a positive correlation ($r = 0.22$, $p < 0.001$), indicating that those who experience more stressful life events also experience more anxiety, and as MZ twins are identical in terms of their genetics and shared environment, this association can be interpreted as truly environmental. To get an indication for the SF-36 scales, we ran the same analysis using the general health domain. We found a negative correlation ($r = -0.09$, $p = 0.04$), indicating that those who experience more stressful life events also report a lower general health-related quality of life. Though it is worth noting that these correlations only explain a small amount of variance ($<5\%$). It may also be worth investigating other factors e.g. physical comorbidities that may explain the covariance between anxiety-physical health.

Strengths

A major strength of our study is the use of a large, representative population-based twin registry based in Sri Lanka, especially considering the limited twin studies in non-west-ern populations. In addition, we used a widely accepted quantitative measure of anxiety symptoms, which not only provides gain in statistical power (Gottschalk and Domschke 2017), but is in line with recent efforts to capture the dimensional nature of traits. As opposed to a categorical approach, symptoms may better conceptualize the anxiety spectrum, help identify those at risk especially individuals that do not meet criteria for a diagnosis (Keough et al. 2010). Our sample also included comparable singletons, further improving power, and was split by sex, revealing significant sex differences in both the aetiology and associations between anxiety and QoL.

Limitations + future directions

We employed a cross-sectional design and are therefore limited in drawing inferences on causality. Higher anxiety may reduce QoL or a poor QoL may in turn increase anxiety

symptoms. A longitudinal twin design and/or extensions to the twin model can better disentangle direction of effects and determine whether genetic and environmental influences increase or attenuate over time (Kendler et al. 2008). Tracking developmental changes in anxiety and QoL can also be useful for designing interventions at the appropriate time. In addition, self-report questionnaires, though commonly used, gauge the presence of anxiety symptoms and perceived QoL rather than establish any diagnoses. Our study should therefore be extended to clinical populations and worth replicating in other South Asian samples to test whether these effects are generalisable. In addition, we did not yield qualitative sex differences, except for marginal (albeit less reliable) effects for C influences in the anxiety- pain analyses. Overall, the same genes and environments seem to be operating across men and women, only with different magnitudes of effect. We may, however, have been underpowered to detect qualitative sex differences, hence future work using larger sample sizes may provide sufficient statistical power to detect this. Furthermore, we find that twins differ from singletons on anxiety and several health related QoL scales even after accounting for age differences. This is also a characteristic of the main COTASS sample (Jayaweera et al. 2018) and might indicate that the twin modelling results may not necessarily extrapolate to the general Sri Lankan population. Additionally, we have attempted to characterise QoL through eight domains, which may not capture its full complexity. Findings must therefore be interpreted within the context of these specific domains rather than generalising to overall QoL. It is also worth noting that confidence intervals (particularly surrounding aetiological correlations) are wide. Replication in larger sample sizes can improve precision in estimates and in drawing more reliable conclusions. In addition, although we did find a large contribution of unique environmental influences, this component also includes measurement error. As we ran bivariate models, correlated measurement error is also worth noting. Language barriers could normally influence this, although we overcame this by ensuring that participants had sufficient language proficiency to take part. As with other studies, however, response bias could affect questionnaire reporting and potentially inflate the non-shared environmental influence. Studies conducted in western populations using the SF-36 and SF-12 scales also find large influence of the unique environment (Romeis et al. 2005; Steenstrup et al. 2013), suggesting that this may be a limitation of the scale. Our findings should therefore be viewed in context of potential measurement error.

One of the most important future application for research of this kind is to inform healthcare planning, to form integrated healthcare systems for mental and physical health (Thornicroft et al. 2019). There are already various barriers to identifying and preventing anxiety in primary care, including stigma, masking/diagnostic overshadowing and prioritising physical diagnoses (Barnes et al. 2019). Screening for both mental, physical and QoL domains in primary care offers a holistic approach, recognising and preventing health issues as early as possible (Firth et al. 2019).

In conclusion, severity of anxiety symptoms was significantly associated with poorer health related quality of life. We find significant sex differences in both the variance and covariance of these traits. For women, individual differences in anxiety and QoL measures were explained largely by shared and unique environmental factors whereas men mostly show evidence of unique environmental influence. In terms of covariances between anxiety and QoL, we find significant overlapping common environmental correlations in females, suggesting the importance of the environment shared, e.g. within families. The unique environment experienced by an individual (including measurement error) had a large contribution to trait variances as well as covariances across sex. Our study is a first considering a behaviour genetic approach combining anxiety and QoL in a south Asian context. Findings have implications for cross-cultural behaviour genetic research and indicate the importance of therapeutic interventions focusing on the wider environment of an individual.

Compliance with ethical standards

Participants were provided with information about the study, including their rights as participants, and provided informed consent.

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Supplementary material for Chapter 4

Supplementary Figure 1. Distribution of GAD-7 anxiety scores in the study sample
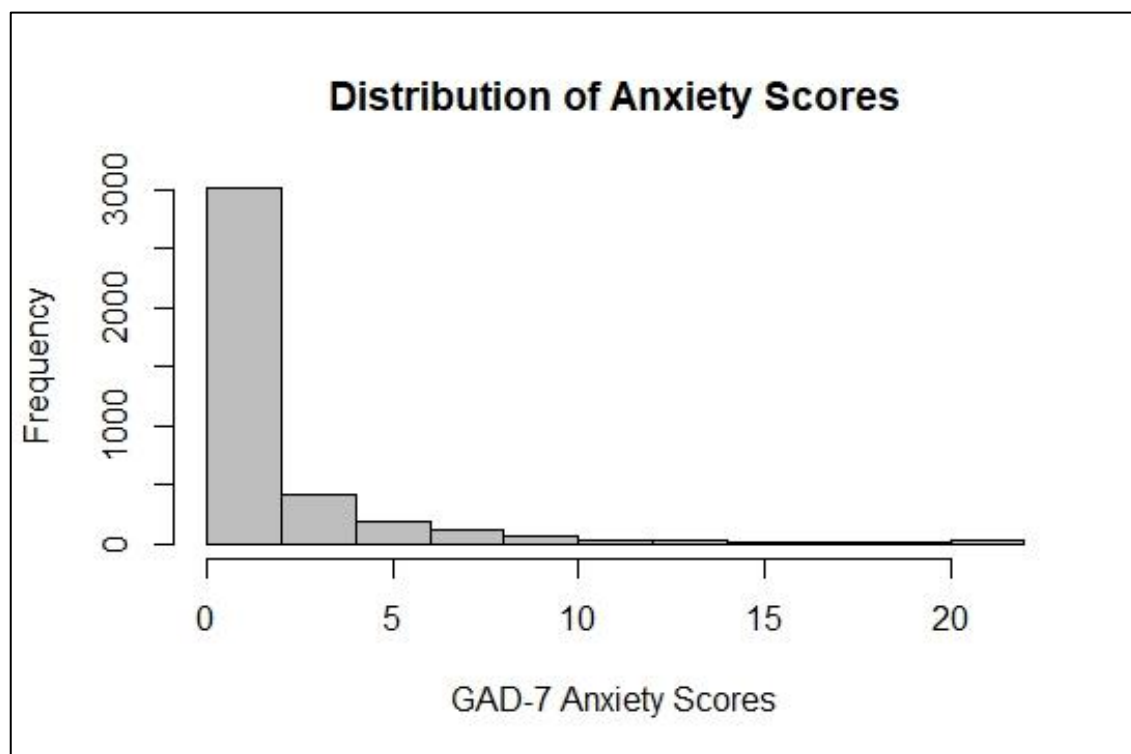
Please note that the GAD-7 anxiety measure was log transformed prior to analyses to minimise skewness. Before transformation: Skewness = 3.02, Kurtosis = 10.87. After transformation: Skewness = .82, Kurtosis = .04.

Supplementary Table I. Welch's t-tests for anxiety symptoms and SF-36 scales.

Variable	Mean Males	Mean Females	t	df	p	95%CI
GAD-7	1.54	2.10	-5.40	3852.3	**<.001**	-.76 , -0.36
GENERAL HEALTH	62.46	59.62	5.54	3757.9	**<.001**	1.83, 3.85
PHYSICAL FUNCTIONING	92.28	86.93	9.03	3822.1	**<.001**	4.18, 6.51
ROLE OF PHYSICAL PROBLEMS	85.13	79.67	4.82	3755.2	**<.001**	3.24, 7.68
EMOTIONAL WELLBEING	79.87	76.88	5.93	3772.6	**<.001**	2.00, 3.98
ROLE OF EMOTIONAL PROBLEMS	89.40	85.18	4.30	3797.2	**<.001**	2.30, 6.14
ENERGY/FATIGUE	73.99	72.81	2.17	3645.5	**.03**	.11, 2.25
PAIN	88.73	85.11	5.63	3741.6	**<.001**	2.36, 4.88
SOCIAL FUNCTIONING	90.14	88.69	2.32	3642.8	**.02**	.23, 2.68

Supplementary Table II. Cross-twin cross-trait correlations between Anxiety symptoms and health related QoL measures for each of the sex-by-zygosity twin groups (with 95% CIs)

QoL VARIABLE	MZM	DZM	MZF	DZF	DZOS
GENERAL HEALTH	.00 (-.10 / .10)	-.06 (-.19 / .08)	**-.14 (-.21 / -.06)**	**-.13 (-.21 / -.04)**	.01 (-.07 / .09)
PHYSICAL FUNCTIONING	-.09 (-.22 /.05)	-.07 (-.21 / .08)	**-.09 (-.16 / -.01)**	**-.16 (-.24 / -.06)**	.01 (-.07 / .09)
ROLE OF PHYSICAL PROBLEMS	-.05 (-.17 / .06)	-.08 (-.21 / .08)	**-.12 (-.19 / -.03)**	**-.15 (-.24 / -.06)**	-.01 (-.09 / .06)
EMOTIONAL WELLBEING	-.09 (-.20 / .02)	-.14 (-.28 / .01)	**-.24 (-.32 / -.15)**	**-.22 (-.31 / -.12)**	-.01 (-.09 / .08)
ROLE OF EMOTIONAL PROBLEMS	-.09 (-.20 / .02)	-.10 (-.25 / .07)	**-.19 (-.27 / -.11)**	**-.26 (-.35 / -.17)**	-.06 (-.14 / .01)
ENERGY/FATIGUE	-.07 (-.18 / .04)	-.04 (-.18 / .12)	**-.22 (-.29 / -.13)**	**-.21 (-.30 / -.11)**	-.05 (-.13 / .02)
PAIN	-.06 (-.16 / .05)	**-.18 (-.30 / -.04)**	**-.13 (-.20 / -.05)**	**-.19 (-.27 / -.10)**	.00 (-.08 / .08)
SOCIAL FUNCTIONING	-.12 (-.25 / .01)	**-.26 (-.41 / -.09)**	**-.22 (-.31 / -.12)**	**-.25 (-.36 / -.14)**	-.08 (-.17 / .02)

MZM = monozygotic male twins; MZF = monozygotic female twins; DZM = dizygotic male twins; DZF = dizygotic female twins; DZOS = dizygotic opposite sex twins. Significant correlations are given in bold (indicated by 95% CI not crossing zero).

Please note that although the cross-twin within trait MZ/DZ twin correlations for general health in males (Table 2 main text) have a 1:1 ratio (indicating that trait aetiologies should largely be due to common environmental effects), we find a significant heritability for males. This is most likely due to the DZOS twins in the model (note that the DZOS twin correlation is small and non-significant), causing a shift in the mean estimates for the correlation in DZ males, leading to a larger difference in the MZ/DZ ratio and therefore a significant heritability estimate. In contrast, the cross-twin cross-trait MZ/DZ correlations (as seen here) for females are significant and have a ratio of 1:1, meaning that the familial effect explaining covariance between Anxiety and these traits is the shared (family) environment, which is what we also see in the results of the ACE model. The mostly non-significant cross-twin cross-trait correlations in males indicate 'E' to be the source of covariance between Anxiety and these traits.

Supplementary Table III. Phenotypic correlations between anxiety symptoms and health-related quality of life (QoL) variables in males and females (95% CIs)

QoL VARIABLE	Sex	Phenotypic Correlation (95%CI)
GENERAL HEALTH	M	**-.29** **(-.33 / -.25)**
	F	**-.26** **(-.30 / -.22)**
PHYSICAL FUNCTIONING	M	**-.21** **(-.26 / -.17)**
	F	**-.17** **(-.21 / -.13)**
ROLE OF PHYSICAL PROBLEMS	M	**-.27** **(-.31 / -.22)**
	F	**-.26** **(-.30 / -.22)**
EMOTIONAL WELLBEING	M	**-.52** **(-.56 / -.49)**
	F	**-.58** **(-.61 / -.55)**
ROLE OF EMOTIONAL PROBLEMS	M	**-.43** **(-.47 / -.39)**
	F	**-.46** **(-.49 / -.42)**
ENERGY/FATIGUE	M	**-.40** **(-.44 / -.36)**
	F	**-.44** **(-.47 / -.41)**
PAIN	M	**-.31** **(-.35 / -.26)**
	F	**-.29** **(-.33 / -.25)**
SOCIAL FUNCTIONING	M	**-.43** **(-.48 / -.38)**
	F	**-.44** **(-.49 / -.40)**

M = Males, F = Females. Significant correlations are given in bold (as indicated by 95% CI not crossing zero).

Supplementary Table IV. A, C and E correlations between Anxiety symptoms and health related QoL measures (with 95% CIs)

QoL VARIABLE	Sex	Correlations with anxiety (95% CI)		
		rA	rC	rE
GENERAL HEALTH	M	-.05 (-.99 / 1)	-.99 (-.99 /-.99)	**-.34 (-.45 / -.23)**
	F	-1 (-1 / -1)	**-.45 (-1 / -.18)**	**-.17 (-.25 / -.09)**
PHYSICAL FUNCTIONING	M	-.99 (-.99 / -.99)	.15 (-1 / .99)	**-.14 (-.26 / -.02)**
	F	-.12 (-.99 / .74)	-1 (-1 / -1)	**-.11 (-.20 / -.01)**
ROLE OF PHYSICAL PROBLEMS	M	-.63 (-1 / 1)	.99 (.99 / .99)	**-.24 (-.35 / -.13)**
	F	.99 (.99 / .99)	**-.49 (-1/ -.26)**	**-.17 (-.25 / -.09)**
EMOTIONAL WELLBEING	M	-.99 (-.99 / .99)	-1 (-1 / -1)	**-.50 (-.57 / -.43)**
	F	-.99 (-.99 / -.99)	**-.99 (-.99 / -.57)**	**-.49 (-.55 / -.42)**
ROLE OF EMOTIONAL PROBLEMS	M	-.84 (-1 / 1)	-.99 (-.99 / -.99)	**-.38 (-.48 / -.28)**
	F	.99 (.99 / .99)	**-.88 (-.99 / -.69)**	**-.32 (-.39 / -.25)**
ENERGY/FATIGUE	M | F	-1 (-1 / -1)	-1 (-1 / -1)	**-.35 (-.41 / -.29)**
PAIN	M	-.99 (-.99 / -.99)	-1 (-1 / -1)	**-.26 (-.36 / -.17)**
	F	-.16 (-.99 / .99)	-.99 (-.99 / -.99)	**-.20 (-.28 / -.11)**
SOCIAL FUNCTIONING	M	-1 (-1 / 1)	-1 (-1 / 1)	**-.38 (-.52 / -.24)**
	F	-.60 (-1 / 1)	-.78 (-1 / 1)	**-.32 (-.44 / -.20)**

M= Males; F= Females. rA = Genetic correlation; rC= Common environmental correlation; rE= Unique environmental correlation. Significant correlations are given in bold (as indicated by 95% CI not crossing zero). Note that these estimates are obtained from the best fitting bivariate ACE model .Also note that there is one set of aetiological correlations for the anxiety-energy/fatigue analysis as this was set to be a hybrid ACE model specifying a homogeneity model for the energy/fatigue scale.

Supplementary Table V. Univariate model fit statistics

Variable	Model	ep	-2LL	df	AIC	ΔLL	Δdf	p
ANXIETY	Sat	9	11892.76	3842	4208.76	-	-	-
	Sub1	8	12028.61	3843	4342.61	135.84	1	2.16E-31
	HetACE	**8**	**11893.64**	**3843**	**4207.64**	**-**	**-**	**-**
	ScACE	6	11906.77	3845	4216.77	13.13	2	<.01
	HomACE	5	12038.59	3846	4346.59	144.95	3	3.24E-31
GENERAL HEALTH	Sat	9	14416.67	3847	6722.67	-	-	-
	Sub1	8	14441.33	3848	6745.33	24.66	1	6.84E-07
	HetACE	**8**	**14418.50**	**3848**	**6722.50**	**-**	**-**	**-**
	ScACE	6	14425.37	3850	6725.37	6.86	2	.03
	HomACE	5	14448.76	3851	6746.76	30.25	3	1.22E-06
PHYSICAL FUNCTIONING	Sat	9	11809.19	3847	4115.19	-	-	-
	Sub1	8	11840.96	3848	4144.962	31.77	1	1.73E-08
	HetACE	8	11814.39	3848	4118.39	-	-	-
	ScACE	**6**	**11818.52**	**3850**	**4118.52**	**4.12**	**2**	**.13**
	HomACE	5	11851.09	3851	4149.09	36.69	3	5.34E-08
ROLE OF PHYSICAL PROBLEMS	Sat	9	20608.09	3847	12914.09	-	-	-
	Sub1	8	20626.69	3848	12930.69	18.60	1	1.61E-05
	HetACE	**8**	**20610.31**	**3848**	**12914.31**	**-**	**-**	**-**
	ScACE	6	20622.93	3850	12922.93	12.62	2	<.01
	HomACE	5	20639.55	3851	12937.55	29.24	3	1.99E-06
EMOTIONAL WELLBEING	Sat	9	14415.11	3847	6721.11	-	-	-
	Sub1	8	14432.29	3848	6736.29	17.18	1	3.41E-05
	HetACE	8	14415.39	3848	6719.39	-	-	-
	ScACE	**6**	**14417.66**	**3850**	**6717.66**	**2.27**	**2**	**.32**
	HomACE	5	14434.05	3851	6732.05	18.65	3	<.01
ROLE OF EMOTIONAL PROBLEMS	Sat	9	14263.04	3847	6569.04	-	-	-
	Sub1	8	14284.18	3848	6588.18	21.14	1	4.27E-06
	HetACE	**8**	**14265.77**	**3848**	**6569.77**	**-**	**-**	**-**
	ScACE	6	14272.17	3850	6572.17	6.40	2	.04
	HomACE	5	14292.23	3851	6590.23	26.46	3	7.63E-06
ENERGY/ FATIGUE	Sat	9	11752.72	3847	4058.72	-	-	-
	Sub1	8	11754.83	3848	4058.83	2.11	1	0.15

	HetACE	8	11752.96	3848	4056.96	-	-	-
	ScACE	**6**	**11754.86**	**3850**	**4054.86**	**1.90**	**2**	**.39**
	HomACE	5	11756.50	3851	4054.50	3.54	3	.32
PAIN	Sat	9	17986.93	3848	10290.93	-	-	-
	Sub1	8	18002.20	3849	10304.20	15.26	1	9.35E-05
	HetACE	8	17994.79	3849	10296.79	-	-	-
	ScACE	**6**	**17997.04**	**3851**	**10295.04**	**2.25**	**2**	**.33**
	HomACE	5	18012.94	3852	10308.94	18.15	3	<.01
SOCIAL FUNCTIONING	Sat	9	4185.07	3888	-3590.93	-	-	-
	Sub1	8	4185.61	3889	-3592.39	0.54	1	.46
	HetACE	**9**	**4187.21**	**3890**	**-3592.79**	**-**	**-**	**-**
	HomACE	6	4375.23	3893	-3410.77	188.02	3	1.63E-40

Note that the homogeneity model was compared to the heterogeneity ACE model. The scalar model (testing differences in variance across sex) was compared to the heterogeneity ACE model. No scalar model was fitted for the social functioning variable as we fitted a liability threshold model with unit variance for males and females. Best fitting models are indicated in bold.

Supplementary table VI. Bivariate model fit statistics

Variable	Model	ep	-2LL	df	AIC	ΔLL	Δdf	p
GENERAL HEALTH	Sat	25	26014.53	7682	10650.53	-	-	-
	Qual A	26	26017.43	7681	10655.43	-	-	-
	Qual C	26	26015.26	7681	10653.26	-	-	-
	HetACE	**22**	**26017.45**	**7685**	**10647.45**	.02	4	.99
						2.18	4	.70
	HomACE	13	26206.65	7694	10818.65	189.20	9	6.05E-36
PHYSICAL FUNCTIONING	Sat	25	23557.57	7682	8193.57	-	-	-
	Scalar Qual A	24	23567.79	7683	8201.79	-	-	-
	Scalar Qual C	24	23566.40	7683	8200.40	-	-	-
	Scalar HetACE	**20**	**23573.61**	**7687**	**8199.61**	5.81	4	.21
						7.21	4	.13
	HomACE	14	23723.84	7693	8337.84	150.23	6	6.91E-30
ROLE OF PHYSICAL PROBLEMS	Sat	25	32231.36	7682	16867.36	-	-	-
	Qual A	26	32235.04	7681	16873.04	-	-	-
	Qual C	26	32233.94	7681	16871.94	-	-	-
	HetACE	**22**	**32235.05**	**7685**	**16865.05**	<.001	4	.99

						1.11	4	.89
	HomACE	13	32414.89	7694	17026.89	179.84	9	5.47E-34
EMOTIONAL WELLBEING	Sat	25	24898.46	7682	9534.46	-	-	-
	Scalar Qual A	24	24901.51	7683	9535.51	-	-	-
	Scalar Qual C	24	24900.35	7683	9534.35	-	-	-
	Scalar HetACE	**20**	**24905.34**	**7687**	**9531.34**	3.83	4	.43
						4.99	4	.29
	HomACE	14	25055.10	7693	9669.10	149.76	6	8.71E-30
ROLE OF EMOTIONAL PROBLEMS	Sat	25	25305.41	7682	9941.41	-	-	-
	Qual A	26	25308.27	7681	9946.27	-	-	-
	Qual C	26	25307.90	7681	9945.90	-	-	-
	HetACE	**22**	**25309.09**	**7685**	**9939.09**	.83	4	.93
						1.20	4	.88
	HomACE	13	25478.03	7694	10090.03	169.77	9	1.03E-31
ENERGY/ FATIGUE	Sat	25	22887.11	7682	7523.11	-	-	-
	Scalar Qual A	20	22896.19	7687	7522.19	-	-	-
	Scalar Qual C	20	22893.92	7687	7519.92	-	-	-
	HetACE	**16**	**22899.55**	**7691**	**7518.55**	4.36	4	.36
						6.62	4	.16
	HomACE	13	23043.55	7694	7655.55	144.98	6	8.50E-31
PAIN	Sat	25	29520.08	7683	14154.08	-	-	-
	Scalar Qual A	24	29533.35	7684	14165.35	-	-	-
	Scalar Qual C	24	29527.35	7684	14159.35	-	-	-
	Scalar HetACE	**20**	**29536.74**	**7688**	**14160.74**	3.39	4	.50
						9.39	4	.05
	HomACE	14	29687.87	7694	14299.87	151.13	6	4.45E-30
SOCIAL FUNCTIONING	Sat	25	16068.17	7723	622.17	-	-	-
	Qual A	28	16073.10	7722	629.10	-	-	-
	Qual C	28	16070.54	7722	626.54	-	-	-
	HetACE	**24**	**16077.83**	**7726**	**625.83**	4.73	4	.32
						7.29	4	.12
	HomACE	15	16223.18	7735	753.18	145.35	9	8.11E-27

Sat = Saturated phenotypic model; Qual A = Model testing for quantitative and qualitative sex differences in genetic influences ; Qual C = Model testing quantitative and qualitative sex differences in shared environmental influences; HetACE= Heterogeneity ACE model testing for quantitative sex differences only (magnitude of genetic and environmental influences on and across variables differs across sex); HomACE= Homogeneity ACE model (genetic and environmental influences on and across variables equated across sex). Note that the HetACE model was compared to the qualitative models (in the order of Qual A then Qual C respectively) and the homogeneity model was compared to the HetACE model. Also note that for variables physical functioning, emotional wellbeing, pain and energy/fatigue a hybrid scalar bivariate model was fit, to allow for variance differences (but no standardized ACE differences) in these variables and aetiological sex differences for anxiety. Ep= estimated parameters; -2LL= -2 Log Likelihood; df= degrees of freedom; AIC = Akaike's Information Criterion (lower values indicate a better fit) ; ΔLL= Difference in -2log likelihood; Δdf= Difference in degrees of freedom; p= p-value. Best-fitting models are indicated in bold.

<u>Supplementary table VII.</u> C correlations obtained from the Qualitative C model for the anxiety – pain analyses indicating marginal qualitative sex differences in C (with 95% CIs)

	PAIN
Rco11 C1m – C1f	.20 (-.61 / .86)
Rco21 C1m – C2f	-.18 (-.99 / 1)
Rco12 C2m – C1f	.11 (-.50 / .78)
Rco22 C2m – C2f	.16 (-1 / 1)

Rco11 = male-female correlation between the C factors of the SF-36 variable, C1m-C1f. Rco21 = correlation between the C factors of the SF-36 variable in males and the anxiety in females , C1m-C2f. Rco12 = correlation between the C factors of anxiety in males and SF-36 variable in females , C2m-C1f. Rco22 = male-female correlation between the C factors of anxiety, C2m-C2f. These correlations are obtained from the Qualitative C model which indicated sex differences in C. Correlations are non-significant, indicated by the 95% confidence intervals crossing zero.

Summary of chapter 4

This chapter explored the relationships between anxiety symptoms and eight areas of health-related quality of life. Results indicate significant and negative phenotypic correlations between these, as well as evidence for aetiological sex differences. Women were more likely to show shared environmental influences both in the variance and covariance of these traits, whereas for men, there was more evidence of some genetic and mostly unique environmental effects. Given that this chapter provided a correlational approach, the next chapter uses the direction-of-causation twin design to explore likely direction of effects between psychological distress and physical health in the same Sri Lankan population.

Chapter 5 . Causal links between psychological distress and physical health

The following is an exact copy of a manuscript currently under review.

Supplementary material can be found at the end of the chapter.

The Causal Link Between Psychological Distress And Physical Health

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Abstract

Background

Psychological distress is associated with poorer physical health, yet the causal relationship between the two domains is not thoroughly understood, especially in non-western populations.

Methods

We use the direction-of-causation (DOC) twin model to investigate this relationship between psychological distress and physical health using cross-sectional, genetically informative data from the Colombo Twin and Singleton Study (COTASS). A DOC model fits reciprocal causal paths between two correlated variables and tests for unidirectional and reciprocal causation. The fit of this model is compared to a correlated-factors model. Self-report measures were used to construct out two latent factors: psychological distress (Anxiety, Depression, Somatic Distress, Emotional wellbeing, and role limitations due to emotional problems) and physical health (General health, physical functioning, role limitations due to physical health problems and pain).

Results

The factors showed a strong negative correlation (r= -.54, 95%CI = -.58, -.50). The fit of a DOC model with reciprocal causal paths between the factors indicated a better explanation of the data compared to a correlated-factors model. A unidirectional model whereby psychological distress may be the cause of poor self-reported physical health showed slightly

more support than the reverse causal relationship.

Limitations

Further research on larger samples is required to confirm this direction of effect.

Conclusions

These results have clinical implications for addressing mental health concerns before they affect individuals' physical wellbeing.

Keywords: Psychological Distress, Physical Health, Twin Study, Causation

Abbreviations:

DOC: Direction of Causation

COTASS: Colombo twin and Singleton Study

MZ: Monozygotic

DZ: Dizygotic

Introduction

Mental and physical health conditions systematically co-occur (Prince et al., 2007), affect people of all ages and come at a significant cost both at individual and economic levels (Firth et al., 2019). Individuals with anxiety and depressive disorders are known to have poorer health outcomes, including chronic conditions such as heart disease and arthritis (El-Gabalawy et al., 2011; Härter et al., 2003; Pinquart and Shen, 2011; Scott et al., 2016), a general decrease in physical functioning and more pain reporting (Stanton et al., 2019). Psychological distress also features somatic symptoms, associated with impaired health status (Creed et al., 2012). Taken together, health-related quality of life is impaired in those with mental health difficulties (Cho et al., 2019; Johansson et al., 2013). Psychological and emotional wellbeing, on the other hand, has been shown to have a protective effect on incidence and progression of physical health problems (Hernandez et al., 2018).

Much of the previous work, however, is cross-sectional or correlational meaning that causality cannot be inferred. It is therefore unclear whether pre-existing physical conditions cause poor psychological adjustment or vice versa. The gold standard for establishing causality have been longitudinal designs and/or randomised control trials. An early longitudinal study suggests no association between health status (including perceived health, chronic diseases and functional limitations) and anxiety outcome over time (Schuurmans et al., 2005) whereas a later study indicated bidirectional relationships between anxiety disorders and physical conditions, including cardiovascular disease and gastrointestinal problems (El-Gabalawy et al., 2014). Other studies also support the notion of bi-directional associations between mental and physical health difficulties (Doherty and Gaughran, 2014;

Naylor et al., 2016; Nuyen et al., 2021; Tegethoff et al., 2016; Yan et al., 2015). Overall, previous research suggests a complex reciprocal interaction but remains inconclusive on causation.

An alternative statistical approach is an extension on the multivariate twin model, the direction of causation (DOC) twin design (Duffy and Martin, 1994; Heath et al., 1993). This model uses cross-sectional, genetically informative data to infer causal inference between two traits, identifying likely direction of effects. More specifically, it uses differences in predicted variances and covariances when the direction of causation is reversed to infer the model that best supports the correlational structure of the data. The power to do so is enhanced when variables (in this case latent factors) have different aetiologies (i.e., one has genetic and one shared environmental sources of variances) or the same aetiology but different in magnitude (i.e., significantly different proportions of genetic and environmental variance components).

The twin DOC model has been used to investigate associations between psychological distress and parenting behaviour, whereby the model suggested that the direction of effects suggest psychological distress is causally associated with parenting behaviour rather than the opposite direction of effects (Gillespie et al., 2003). This model has also been used to examine the relationship between internalising symptoms and sleep (Gillespie et al., 2012). As of yet, however, no DOC study has investigated likely causal directions of effect between psychological distress and measures of physical health.

Previous research has largely focused on High Income Western populations and there is a lack of representation in non-western populations. Research that has been conducted in non-

European populations found associations between anxiety-depression and self-rated health and wellbeing in Bangladesh (Hossain et al., 2020) and China (Malone and Wachholtz, 2018). Yet, there is still limited behaviour genetic work in non-western samples, especially in understanding causal links. In this paper, we investigate the causal direction of the relationship between physical and psychological health using cross-sectional, genetically informative data from the Sri Lankan twin registry. To account for measurement error,an important requirement to detect unbiased direction of causal effects, we constructed two latent factors from self-reported measures on: 1) Psychological distress (Anxiety, Depression, Somatic Distress, Emotional wellbeing, and Role limitations due to emotional problems) and 2) Physical health (General health, physical functioning, role limitations due to physical health problems, and pain). Based on previous work suggesting bi-directional relationships between mental-physical health domains (Doherty and Gaughran, 2014; Naylor et al., 2016), we hypothesise a reciprocal interaction between the two factors.

Methods

Sample

The Colombo Twin and Singleton Study (COTASS) is a representative population-based sample as part of the Sri Lankan twin registry (Siribaddana et al., 2008; Sumathipala et al., 2013). In this study, we used data from the second phase (COTASS-2) (Jayaweera et al., 2018), with twins (N=2922) followed up from the original study through invitation letters, telephone interviews and home visits. Informed written consent was obtained from all participants. Individuals were excluded if they did not understand the consent procedure or the questionnaires due to language barriers. Participants that completed one or more study

parts were offered 750 LKR (approximately £3.50 GBP) reimbursement for their time. The study has received ethical approval from the ethics committee at the Faculty of Medical Sciences, University of Sri Jayewardenepura, Sri Lanka (reference number: 596/11) and the Psychiatry, Nursing & Midwifery Research Ethics subcommittee, King's College London, UK (reference number: PNM/10/11-124).

Measures

To be able to construct two factors that were as close as possible to being purely 'psychological' and purely 'physical', we firstly conducted a principal components analysis (PCA) with varimax rotation on items available from four administered questionnaires. Due to our aims and the nature of causation analysis, we specified a two-factor solution, with one factor signifying 'psychological distress' and the other representing 'physical health'. Specific item loadings on these two factors are detailed in **Supplementary material 1**. Based on item loadings, we have chosen to have 9 variables in total loading onto the two latent factors, summarised below.

Psychological distress

We used items from four questionnaires to construct this latent common factor. First, anxiety symptoms measured using the *GAD-7* (Spitzer et al., 2006), capturing the presence of generalised anxiety, over the past 2 weeks. The 7-item questionnaire asks participants to rate how often they feel bothered by problems such as 'feeling nervous, anxious or on edge' and 'trouble relaxing', from 0 (Not at all) to 3 (nearly every day). Since all of these items loaded on the psychological distress factor, we used the total anxiety score, ranging from 0 to 21.

Second, depressive symptoms measured using the 21-item *Beck's Depression Inventory (BDI)* (Beck et al., 1961). Participants were asked to self-rate how they feel on items such as 'I feel sad' with a scale ranging from 0 (I do not feel sad) to 3 (I am so sad and unhappy that I can't stand it). Items 15, 20 and 21, however showed a poor loading to the psychological distress factor and were therefore omitted. The new total depression score ranged from 0-47 in the sample.

Third, somatic distress associated with anxiety and depression as measured by the *Bradford Somatic Inventory (BSI)* (Mumford et al., 1991). The measure is a 21-item questionnaire asking questions such as 'Have you been aware of palpitations (heart pounding)?' with a scale ranging from 0 (Absent), 1 (Present on < 15 days during last month) and 2 (Present for > 15 days during last month). Four out of the 21 items (mostly referring to tension headaches) loaded on the psychological distress factor and were included as a sum score, ranging from 0-8.

The fourth and fifth variables loading on the psychological distress factor were *emotional wellbeing* (as measured by five items) and *role limitations due to emotional difficulties* (measured by three items), both domain scores as measured with the *Short Form Health Survey* (SF-36), each scale ranging from 0 (e.g., low emotional wellbeing) to 100 (e.g., highest level of emotional wellbeing).

Physical Health

To construct this latent factor, we used items from the *Short Form Health Survey (SF-36)*

(Ware and Sherbourne, 1992). The 36-item measure eight domains of health of which four loaded onto the physical health factor: general health perceptions (five items); physical limitations (physical functioning; 10 items), role limitations due to physical health problems (role physical; 4 items) and bodily pain (two items). Some items on the questionnaire are recoded so that the scores range from 0-100, with 100 representing the best state of health, and 0 indicating worst. After recoding, an average score was obtained for each domain. The SF-36 survey has been widely used, with good reliability and validity (Mchorney et al., 1993) and useful when adapted across cultures (Ahmed et al., 2002).

Analyses

To estimate the correlational structure of the twin data we first fitted a phenotypic correlated factors model. We applied constraints to this model to yield a more constrained output including: one overall phenotypic correlation (rPh) between the two factors, regardless of twin order or zygosity; cross-twin cross-factor correlations for MZ (monozygotic) and DZ (dizygotic) twins, which are independent of twin order (e.g. Psychological distress Twin 1 – Physical health Twin 2 is equal to Physical health Twin 1 – Psychological distress Twin 2); cross-twin within-factor correlations for MZ and DZ twins separately (i.e. MZ and DZ twin correlations for Psychological distress and Physical health). The ratio of these MZ and DZ cross-twin within-factor and cross-factor correlations will indicate the etiological components of (co)variance of the two factors.

Next, we follow principles of the classical twin design to fit models with latent genetic and environmental factors. This is based on the comparison of resemblance between monozygotic

(identical) twins who share 100% of their DNA, and dizygotic (non-identical) twins who share, on average, 50% of their segregating genes. Using the information from the MZ and DZ cross-twin within-trait correlations, the phenotypic variance of traits can be decomposed into three latent sources: additive genetic (A), common environmental (C; contributing to similarity within twin pairs), and unique environmental factors (E; contributing to differences within twin pairs, including measurement error). The ratio of MZ:DZ correlations provide information on the extent of familial influence. A ratio of 2:1 indicates the relative influence of genetic effects whereas a 1:1 ratio suggests the role of common environmental influences.

Similarly, in a bivariate model (when we analyse two variables at the same time), covariance between two traits can be disentangled into aetiological correlations (denoted rA, rC and rE) using cross-twin cross trait correlations. This indicates how much A, C and E factors underlying individual differences in one also affects the other (Rijsdijk & Sham 2002). Significant phenotypic correlations between latent psychological distress and physical health factors would imply common aetiology, and significant cross-twin cross-trait correlations suggest that this aetiology is familial. The ratio of the MZ:DZ cross-twin cross-trait correlations indicate to what extent this common aetiology is genetic or environmental in origin; a 2:1 ratio suggests the effects of A, a 1:1 ratio suggests the effects of C and nonsignificant cross- trait cross-twin correlations suggest that the common aetiology is due to E (Neale & Cardon, 2013). Similar steps apply for an overall breakdown of the phenotypic correlation to yield the parts due to correlated A, C and E factors (rPh-A, rPh-C and rPh-E, respectively).

In this study, we extend the classic twin model further in two ways; constructing a full bivariate ACE common factor model (psychological distress and physical health) and using the genetically informative data to fit a direction of causation (DOC) twin model (Duffy and Martin, 1994; Gillespie et al., 2003; Heath et al., 1993). Model-fitting analysis was conducted by 1) fitting the full bivariate ACE correlated factor model, 2) the reciprocal causation model, 3) two unidirectional models, whereby the latent psychological distress factor → the latent physical health factor and then visa-versa, the physical health factor → the psychological distress factor, and finally (4) a no-association model whereby the A, C and E covariance paths as well as the causal paths are fixed to zero. The power of the DOC design rests on the pattern of cross-twin, cross-trait correlations. This is the case when two traits have different variance compositions (e.g., one has and AE and the other a CE model), predicted cross-twin, cross-trait correlations will differ across MZ and DZ twins giving the power to detect/reject causation models. This is also the case when two variables both show ACE influence, but with significantly different proportions of variance components. If, however, two traits have identical modes of variance that are similar in magnitude, cross-twin cross-trait correlations will be equal across MZ and DZ twins, eliminating power to disentangle causation. More details on the DOC model can be found in **Supplementary material 2 & 3**.

Twin model fitting was conducted using the OpenMx statistical package in R (Neale et al., 2016). We used continuous data tapping into psychological distress and physical health, regressed by age and sex and log transformed to minimise skew. To detect the best-fitting model, differences in minus twice log likelihood (-2LL) (distributed as χ^2) were examined between nested models. In addition, the Akaike's Information Criterion (AIC) and Bayesian

Information Criterion (BIC) were evaluated whereby lower values generally indicate a better fit (Neale and Cardon, 2013; Raftery, 1995; Rijsdijk and Sham, 2002). Further guidelines for interpreting differences in AIC and BIC values are reported elsewhere (Burnham and Anderson, 2002; Markon and Krueger, 2004)

Results

Mean age of the sample was 42.84 years (SD= 14.56). Descriptive statistics for variables used (split by zygosity group) is shown in **Table 1**.

Table 1. Means (standard deviations) for variables used to construct the two common factors

Factor	Variable (Range)	Mean (S.D)	
		MZ (N= 1263)	**DZ** (N= 1659)
Psychological Distress	Anxiety (0-21)	1.75 (2.94)	1.76 (3.32)
	Depression (0-47)	3.44 (4.91)	3.64 (5.23)
	Somatic distress (0-8)	1.03 (1.60)	1.06 (1.67)
	Emotional wellbeing (0-100)	77.99 (15.64)	78.49 (15.83)
	Role limitations due to emotional problems (0-100)	86.69 (30.70)	87.87 (15.83)
Physical Health	General health (0-100)	62.21 (15.81)	61.20 (15.90)
	Physical functioning (0-100)	90.74 (17.74)	90.98 (17.13)
	Role limitations due to physical problems (0-100)	83.70 (33.90)	82.77 (34.83)
	Pain (0-100)	86.81 (20.34)	86.40 (19.67)

Note that a score of 100 indicates better functioning on the SF-36 variables.

Phenotypic analyses

Twin correlations for the two factors (psychological distress and physical health) are shown in **Table 2**. The two factors were negatively correlated (r = -.54, 95% CI = -.58, -.50), suggesting that psychological distress is associated with decreased self-reported physical health and vice versa. The cross-twin, within-factor correlations for psychological distress indicates an approximate 2:1 ratio for MZ and DZ twins, suggesting that genetic influence is most likely to explain variance in this factor. On the other hand, the cross-twin within-factor correlation for physical health is roughly a 1:1 ratio for both sets of twins, indicating that variance in the factor is most likely explained by shared environmental effects. Using the same principle, the cross-twin cross-factor correlation for MZ twins is almost twice as large of DZ twins (2:1 ratio), signifying that the covariance (aetiological overlap) between the two factors is mostly explained by genetic influences.

Table 2. Twin correlations within and across factors (95%CI)

	Cross-twin, Within factor		Cross-Twin, Cross Factor	rPh
	Psychological Distress	Physical health		
MZ	.38 (.28 , .47)	.27 (.17 , .37)	-.21 (-.29 , -.14)	-.54 (-.58 , -.50)
DZ	.20 (.12 , .29)	.20 (.11 , .29)	-.12 (-.18 , -.05)	

rPh = Phenotypic correlation between factors. MZ= Monozygotic twin pairs; DZ= Dizygotic twin pairs. All correlations were significant (indicated by 95% CI not spanning zero)

Bivariate ACE Factor analysis

A full bivariate genetic model was fitted to the factor structure of the data, as a foundation for the later DOC models. Standardised variance components, aetiological correlations and the phenotypic correlation breakdown can be found in **Table 3**. We found a significant heritability estimate for the psychological distress factor (33%), being almost twice the heritability of the physical health factor (18%). Common environmental influences were higher for physical health although non-significant for both factors. Unique environmental influences (including measurement error) were high for both factors. Some of the ACE estimates specific to each measured variable were non-significant, details of these effects can be found in **Supplementary material 4**. We did not find significant genetic and shared environmental correlations between the two factors.

Table 3. Standardised variance components of factors, aetiological correlations, and phenotypic correlation (and its genetic and environmental components) (95% CI) from the bivariate ACE model

Factor	Aetiology		
	A	C	E
Psychological distress	**.33 (.08 / .44)**	.02 (.00 / .22)	**.65 (.56 / .74)**
Physical health	.18 (.00 / .37)	.10 (.00 / .26)	**.72 (.63 / .82)**
	Aetiological Correlations		
	rA	rC	rE
	-.84 (-1 / .99)	-.07 (-1 /1)	**-.49 (-.57 / -.40)**
	Phenotypic correlation		

Rph	Rph-A	Rph-C	Rph-E
-.54 (-.57 / -.50)	-.20 (-.32 / .00)	.00 (-.16 / .07)	**-.34 (-.41 / -.26)**

A= Genetic influence/heritability; C= Common environmental influence; E= Unique environmental influence. rA= Genetic correlation; rC= Common environmental correlation; rE = Unique environmental correlation. Rph = Phenotypic correlation as derived from the Bivariate ACE model, Rph-ACE = Phenotypic correlation breakdown into ACE components.

Direction of Causation analysis

Our primary aim was to explore whether a specific causal direction would better fit the data compared to the bi-directional model and whether the bi-directional model could better explain the relationship between the two factors compared to the correlational ACE factor model. This was done by nesting four causal models under the full bivariate ACE model. Goodness of fit statistics for all nested models are shown in **Table 4**. Based on model fit statistics, there was no significant decline in fit upon comparing the bi-directional reciprocal causation model with the full bivariate ACE model. Unidirectional models also did not have a significant reduction in fit, although it is difficult to draw conclusions on any best-fitting unidirectional model. The no-correlation model resulted in a significant decline in fit.

When comparing the unidirectional Psychological Distress → Physical Health model with the reciprocal causal model, the reduction in fit (Δ-2LL = .19, p=.66) is non-significant. However looking at the other fit indices (ΔAIC = 1.81, ΔBIC = 7.22) between the two models, the guidelines would suggest quite a bit of support (Burnham & Anderson, 2002) and strong evidence (Raftery, 1995) for the simpler unidirectional model.

When comparing the unidirectional Physical Health→ Psychological distress model with the reciprocal causal model, the reduction in fit (Δ-2LL = 2.81, p=.09) is also non-significant.

Looking at the difference in the other fit indices (ΔAIC = .81, ΔBIC = 4.6), the more negative

BIC value for the unidirectional model also indicates some support for this simpler model.

In conclusion, based on the likelihood ratio tests, we cannot select any unidirectional model

as best fitting. There is, however, more support for the Psychological Distress → Physical

Health unidirectional model based on both the AIC and BIC indices, and we have therefore

provided a path diagram depicting this model in **Figure 1**.

Table 4. Results of Fitting Direction of Causation Models to the Psychological Distress and
Physical Health Factors

Model	Goodness of fit						
	-2LL	df	Δ -2LL	Δ df	p	AIC	BIC
Full Bivariate ACE	90513.67	25417				39679.67	-97788.96
Reciprocal causation	90513.67	25418	<.01	1	.95	39677.67	-97796.36
Psychological Distress→ Physical Health	90513.86	25419	.19	2	.91	39675.86	-97803.58
Physical Health→Psychological Distress	90516.48	25419	2.81	2	.24	39678.48	-97800.96
No correlation	91069.78	25420	556.11	3	<.001	40229.78	-97255.07

Psychological distress as measured by 5 indicators: Anxiety, Depression, Somatic Distress, Emotional
wellbeing and Role limitations due to emotional problems. Physical health as measured by 4
indicators: General health, Physical functioning, Role limitations due to physical health problems and
Pain. The reciprocal causation, unidirectional, and no correlation models were compared to the full

bivariate ACE model.

Figure. 1 Unidirectional causation factor model for the psychological distress and physical

health variables with standardised path coefficients (95% confidence intervals).
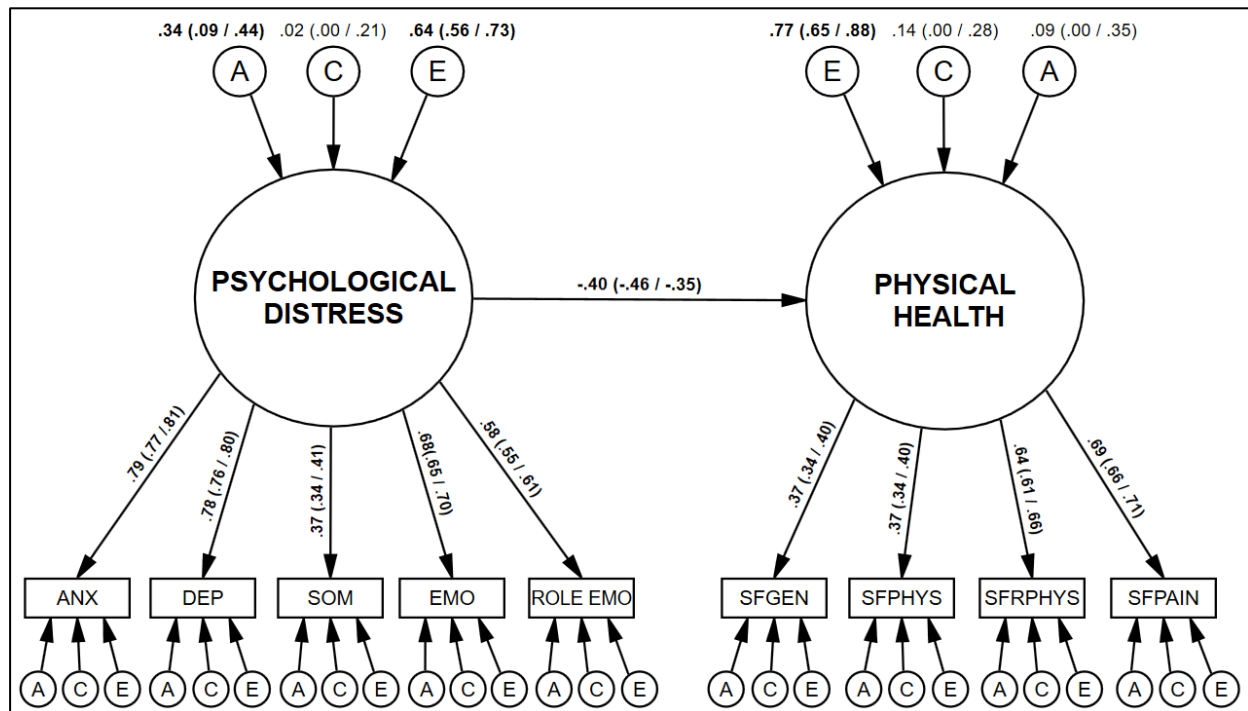

Figure legend. A= Additive genetic effects; C= Common environmental effects; E= Unique environmental effects. ANX = Generalised anxiety symptoms, as measured by the GAD-7; DEP= Depression symptoms as measured by beck's depression inventory; SOM= Somatic distress, as measured by the Bradford Somatic Inventory; EMO= Emotional wellbeing, as measured by the SF-36 health survey; ROLE Emo= Role limitations due to emotional problems as measured by the SF-36 health survey. SFGEN= Perceived general health; SFPHYS= Physical functioning; SFRPHYS= Role limitations due to physical health problems; SFPAIN= Bodily pain. Paths from common factors to measured variables represent factor loadings. Path running from the psychological distress to physical health factor represents the unidirectional causal path. For simplicity, we have not included the specific genetic and environmental estimates on the measured variables in this figure, these are detailed in **Supplementary Table 5**. Note that the A, C and E estimates for Physical Health in this figure are the total effects (summing up to 100%). These include time specific effects as well as the ACE variances transmitted from Psychological Distress via the causal path. Significant paths are specified in bold (indicated by confidence interval not crossing zero).

Discussion

This twin study aimed to test the direction of causation between psychological distress and

physical health factors in a large population-based twin sample. Phenotypically, we find a strong negative correlation between the two latent factors, suggesting an association between psychological distress and physical health. Using bivariate genetic model fitting, we find evidence of heritability for both factors, though only the estimate for psychological distress was significant. Genetic differences within the population therefore explain a significant (albeit modest) proportion of variance in these traits. Heritability of the psychological distress factor (33%) is lower than a similar study on Australian twins (Gillespie et al., 2003) and in other twin studies on mental wellbeing in the US (Keyes et al., 2010), though closer to that from the UK (Rijsdijk et al., 2003). These previous studies, however, use a mixture of variables to construct a psychological distress factor, which can influence these estimates.

The physical health factor had a low heritability (18%), and was lower in comparison to similar studies on self-perceived health status in Finnish and Norwegian samples (Leinonen et al., 2005; Røysamb et al., 2003). Our estimate, is, however, in line with a Danish twin study on physical health-related quality of life where estimates ranged from 11-32% (Steenstrup et al., 2013). The specific heritability estimates for the SF-36 physical health variables are also lower than a previous study on male twins from the US, which ranged 18-33% for the four variables directly used in this study (Romeis et al., 2005). In this study, however, we go beyond previous studies, reporting genetic and environmental (shared & non-shared) correlations between psychological distress and physical health and investigated the causal direction of the relationship.

Through structural equation modelling, we find that a reciprocal causation model explained

the data as well as the full bivariate ACE model. In addition, we find that compared to the reciprocal causation model, there is tentative support for a unidirectional model in which poor psychological health can be a risk factor for physical health problems. In a wider context, these findings are in keeping with studies suggesting the role of mental health problems as a risk factor for physical ill health, and conversely, that health problems can increase risk of poor mental health. Although we do not measure physical illness directly, our study has implications for public health policy in recognising and implementing mental healthcare alongside physical treatment (Firth et al., 2019; Naylor et al., 2016).

Limitations + future directions

Firstly, although there was more evidence for the unidirectional model (psychological distress leading to physical health problems) in terms of lowest AIC and BIC values, the improvement is marginal in comparison to the reciprocal causation model. It is not possible to reject a mutual interaction between psychological distress and physical health nor the correlated ACE model. Nevertheless, we believe the DOC model here provides a potential explanation for physical decline following mental health problems.

Secondly, the DOC model, although an elegant alternate option for understanding causal direction in cross-sectional data, also has its limitations and biases (Duffy and Martin, 1994). One of these is the effect of measurement error (which is accounted for in the unique environmental influence component, E, in twin studies). The large influence of unique environmental effects was also the case for the psychological distress factor and specific E estimates for anxiety, depression and somatic distress in an Australian twin sample (Gillespie

et al., 2003). Twin studies on self-perceived health, however, report lower E influence (Leinonen et al., 2005; Røysamb et al., 2003) although E estimates for the SF-36 variables are in keeping with the US sample (Romeis et al., 2005). Here, we partly overcome this by using several indicators loading on the two common factors, each with their own residual error variances. The latent factors are therefore closer to being 'true' scores/representations of the constructs. The role of non-shared environmental confounding, however, remains an issue with the design and could introduce bias in estimates (Rasmussen et al., 2019).

Although the DOC twin model has been introduced over a decade ago, research has not benefitted from this alternative statistical approach much, especially in non-western populations. This may be due to the relatively low power and the necessity for variables to have different aetiologies or the same aetiology but non-overlapping variance components. Despite these limitations, we believe that with cautious interpretation, the model offers a way to begin disentangling causal influences when genetically informative data is available. Next steps would be to move towards other designs to infer causality such as mendelian randomisation (Davey Smith and Hemani, 2014) using polygenic risk scores and combining these with the DOC twin method (Minică et al., 2018). We do, however, require genotype data to be able to do this, which is yet to be conducted in the COTASS sample.

Thirdly, the two latent factors presented here consist of self-reported, perceived mental and physical wellbeing rather than diagnosed disorders and are not extensive measurements. Although these constructs provide a dimensional approach, are validated, and used previously, it would be worth extending this research to diagnostic measurements to be able

to extrapolate results.

Finally, the association between psychological and physical health is clearly complex. Although understanding the mechanisms in which they are linked is beyond the scope of this paper, we believe these findings and further research can have implications for future prevention and intervention. If psychological distress is a precursor for poor physical health, then it may be worth attending to individuals' mental health issues before physical health problems arise. Similarly, if there is a reciprocal interaction, prioritising and integrating the two domains into healthcare may be a useful approach. Furthermore, understanding the genetic and environmental contribution to the mental-physical health interaction and the direction of effect can better inform on interventions at the appropriate time. Further DOC twin studies, in combination with other methods of understanding causality can formulate a better picture for clinical implementation.

Conclusions

In conclusion, using a population-based twin sample based in Sri Lanka, we find a significant negative association between latent psychological distress and physical health factors. Taking this further, we find some evidence in support of a unidirectional causation model whereby psychological distress may place individuals at risk of poor physical health. Further research in larger, diverse samples are required to clarify this causal link.

Author Contributions: ZN conceived the study and conducted analyses under the supervision of FR. All authors provided critical revision of the manuscript.

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Supplementary material for Chapter 5

1. **Principal component analysis with varimax rotation**

Items included	PC1	PC2	h2/ commonality	u2/ uniqueness	Complexity
Bradford Somatic Inventory					
brad1	0.34	0.21	0.161	0.84	1.7
brad2	0.21	0.23	0.1	0.9	2
brad3	0.22	0.31	0.144	0.86	1.8
brad4	0.34	0.17	0.147	0.85	1.5
brad5	0.25	0.35	0.182	0.82	1.8
brad6	0.28	0.35	0.198	0.8	1.9
brad7	0.32	0.47	0.325	0.68	1.7
brad8	0.13	0.22	0.064	0.94	1.6
brad9	0.23	0.35	0.176	0.82	1.7
brad10	0.21	0.26	0.114	0.89	1.9
brad11	0.27	0.44	0.268	0.73	1.6
brad12	0.2	0.22	0.088	0.91	2
brad13	0.25	0.32	0.162	0.84	1.9
brad14	0.11	0.3	0.099	0.9	1.3
brad15	0.35	0.27	0.196	0.8	1.9
brad16	0.27	0.39	0.226	0.77	1.8
brad17	0.31	0.22	0.145	0.86	1.8
brad18	0.11	0.33	0.124	0.88	1.2
brad19	0.19	0.27	0.106	0.89	1.8
brad20	0.18	0.26	0.103	0.9	1.8
brad21	0.13	0.26	0.084	0.92	1.5
SF-36 items					
sf1	0.21	0.49	0.282	0.72	1.4
sf2	0.19	0.41	0.202	0.8	1.4
sf3	0.03	-0.59	0.353	0.65	1
sf4	0.06	-0.61	0.37	0.63	1
sf5	0.07	-0.68	0.462	0.54	1
sf6	0.01	-0.69	0.471	0.53	1
sf7	0.04	-0.64	0.415	0.59	1
sf8	0	-0.55	0.298	0.7	1
sf9	0.04	-0.69	0.478	0.52	1
sf10	0.08	-0.68	0.469	0.53	1
sf11	0.09	-0.66	0.447	0.55	1
sf12	0.01	-0.46	0.216	0.78	1
sf13	-0.19	-0.61	0.409	0.59	1.2
sf14	-0.19	-0.61	0.413	0.59	1.2

sf15	-0.17	-0.61	0.4	0.6	1.2
sf16	-0.17	-0.62	0.414	0.59	1.2
sf17	-0.49	-0.35	0.361	0.64	1.8
sf18	-0.48	-0.35	0.354	0.65	1.8
sf19	-0.51	-0.3	0.355	0.65	1.6
sf20	0.26	0.59	0.413	0.59	1.4
sf21	0.24	0.55	0.359	0.64	1.4
sf22	0.25	0.63	0.457	0.54	1.3
sf23	0.36	0.38	0.274	0.73	2
sf24	-0.57	-0.15	0.348	0.65	1.1
sf25	-0.6	-0.18	0.391	0.61	1.2
sf26	0.34	0.17	0.145	0.85	1.4
sf27	0.33	0.4	0.272	0.73	1.9
sf28	-0.62	-0.16	0.408	0.59	1.1
sf29	-0.36	-0.22	0.18	0.82	1.6
sf30	0.48	0.26	0.295	0.7	1.6
sf31	-0.32	-0.26	0.171	0.83	1.9
sf32	-0.31	-0.47	0.316	0.68	1.7
sf33	-0.21	-0.4	0.204	0.8	1.5
sf34	0.17	0.42	0.202	0.8	1.3
sf35	-0.17	-0.3	0.12	0.88	1.6
sf36	0.2	0.38	0.183	0.82	1.5
Beck's Depression Inventory					
beck.q1	0.64	0.09	0.417	0.58	1
beck.q2	0.55	0.14	0.32	0.68	1.1
beck.q3	0.51	0.06	0.267	0.73	1
beck.q4	0.64	0.12	0.429	0.57	1.1
beck.q5	0.51	0	0.261	0.74	1
beck.q6	0.51	0.01	0.262	0.74	1
beck.q7	0.6	0.08	0.371	0.63	1
beck.q8	0.49	-0.01	0.241	0.76	1
beck.q9	0.47	0.07	0.222	0.78	1
beck.q10	0.49	0.05	0.242	0.76	1
beck.q11	0.49	0.12	0.252	0.75	1.1
beck.q12	0.41	0.17	0.201	0.8	1.3
beck.q13	0.44	0.2	0.233	0.77	1.4
beck.q14	0.51	0.11	0.275	0.73	1.1
beck.q15	0.34	0.48	0.346	0.65	1.8
beck.q17	0.49	0.16	0.268	0.73	1.2
beck.q19	0.52	0.24	0.329	0.67	1.4
beck.q20	0.32	0.47	0.321	0.68	1.8
beck.q21	0.16	0.33	0.137	0.86	1.4
beck.q16a	0.32	0.25	0.168	0.83	1.9

beck.q18a	0.29	0.29	0.17	0.83	2
GAD-7 anxiety items					
gad.1	0.71	0.08	0.517	0.48	1
gad.2	0.68	0.08	0.469	0.53	1
gad.3	0.69	0.08	0.483	0.52	1
gad.4	0.66	0.14	0.46	0.54	1.1
gad.5	0.58	0.17	0.364	0.64	1.2
gad.6	0.52	0.13	0.292	0.71	1.1
gad.7	0.65	0.1	0.435	0.56	1

	RC1	RC2
SS loadings	12.23	11.64
Proportion Var	0.14	0.14
Cumulative Var	0.14	0.28
Proportion Explained	0.51	0.49
Cumulative Proportion	0.51	1

Mean item complexity = 1.4
Test of the hypothesis that 2 components are sufficient.

The root mean square of the residuals (RMSR) is 0.07
 with the empirical chi square 117032.4 with prob < 0

Fit based upon off diagonal values = 0.91

2. <u>Causal inference using the twin design</u>

Twin studies are not only useful in partitioning variance and covariance of traits but can also be used for causal *inference*. Although not a direct test of causality, the twin method can be useful to understand whether associations between two traits persist after accounting for confounding effects of common aetiology. To illustrate this, we have a hypothetical example of variables X and Y. The association between X and Y cannot be deemed casual (Figure 1a) as it can be confounded by a common causal variable. One way to overcome this is to regress both X and Y on a confounding third variable, Z (Figure 1b). This method, however, is challenging for three main reasons; 1) confounders must be well identified and measured so as to avoid bias in the estimate Byx ; 2) measuring all confounders is difficult (most likely impossible) and 3) even if all confounds are measured and added to models, statistical power would be greatly reduced.

The twin method offers an alternative approach, controlling for latent genetic and environmental factors, without having to actually identify or measure them (Figure 1c). Using the principles of the twin model, covariance between two traits can be decomposed into A (genetic), C (shared environmental) and E (unique environmental) influences. The A factor (correlated 1 for MZ twin pairs and .5 for DZ twin pairs) not only explains variance in trait X but also the covariance between trait X and Y (within and between

twins). Thus, this accounts for genetic confounding between the two traits. The same goes for C influences, where twin-pair resemblance is 1 for both MZ and DZ twin pairs. This accounts for shared environmental confounding between the traits. E is uncorrelated between and within twins, so can only inform on the within-person covariance after controlling for the familial effects of A and C. Hence, A and C control for latent genetic and shared environmental confounders in the same way that variable Z accounts for measured confounds in Figure 1b. The major advantage here is that we do not need to measure all potential confounders or worry about measurement error. Twin data therefore allows control over familial (shared) confounders in twin pairs.
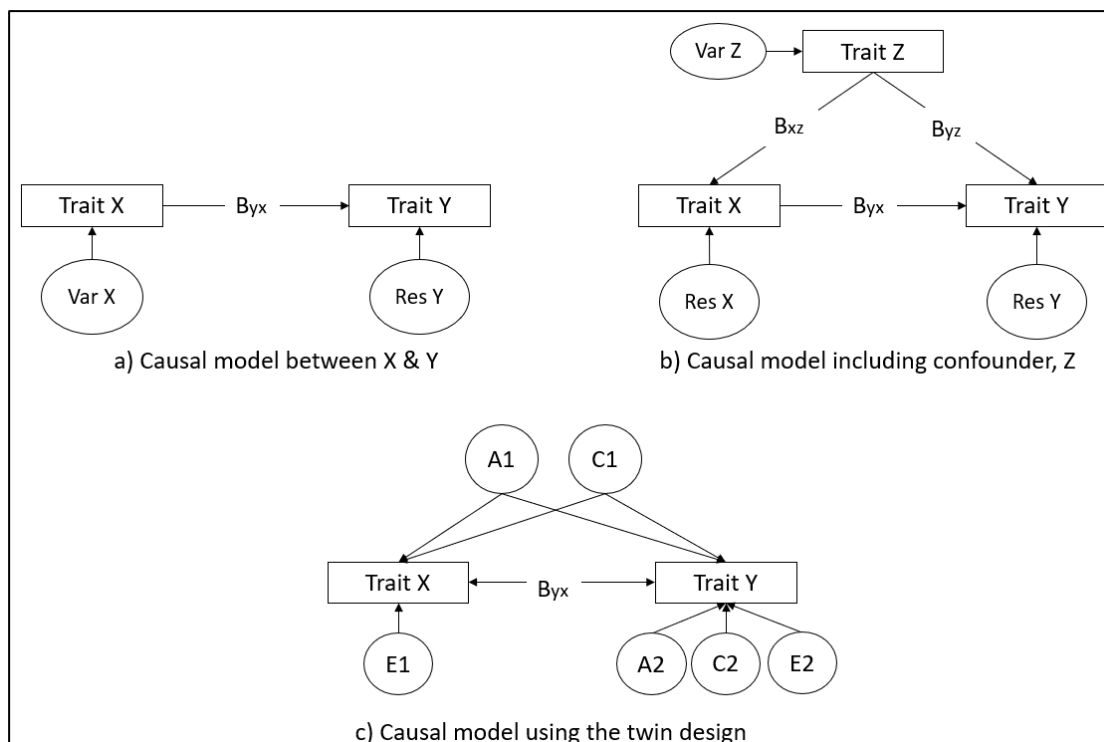

Figure 1. Modelling causality.

Byx represents the causal effect of X on Y. In model a) Var X indicates the variance of X, and Res Y indicates the residual variance of trait Y (the variance of Y remaining after regressing out the effects of X). In model b) Var Z symbolises the variance of Z, and Res X and Res Y denote the residual variances of X and Y (variance left after regressing out the effect of Z (on X) and Z and X (on Y)). In model c) the path Byx indicates a causal path after accounting for the familial (genetic & shared environmental) effects. Note that this twin model is a partial path diagram for a single individual. All latent factors (depicted in circles) have a variance of 1 (not shown).

There are various methods in which twin pairs can be used for causal inference. This includes the Cholesky decomposition partly described above, longitudinal twin models, the co-twin control method, and the children-of-twins design. In this paper, however, we focus on the direction-of-causation design, an alternative to inferring causal direction using cross sectional data. We review the logic of the model below.

3. Direction of Causation (DOC) Twin model

Under certain circumstances, cross-sectional data can be a useful resource for moving closer to causal links between traits. The Direction of Causation (DOC) model (Duffy & Martin, 1994; Heath et al., 1993)is one method. Unlike previously described causation analyses, the focus is not to control for confounding effects of common aetiologies, but to investigate the likely direction of effects. For instance, the extent to which the association between Psychological distress (PD) and Physical health (PH) is driven by PD predicting PH vs. PH predicting PD.

To achieve this, the model relies on differential cross-twin cross-trait correlational structure for MZ and DZ twin pairs, rooted in the differential aetiologies of traits (the relative influence of A, C and E). As an example, let us imagine the two factors in our study, PD being influenced more so by A whereas PH showing more influence of C. We have provided a simplified path diagram of this below. Under the PD causes PH hypothesis (Figure 2a), the cross-twin cross trait correlation can only be explained through variance of PD (following path tracing rules). This means tracing back from the PD twin 1 latent factor, through the double headed arrows connecting the twin pair (genetic covariance) and down to the PH factor for twin 2. The resulting cross-twin cross-trait correlation is therefore calculated as $a11^2 * i1$ for MZ and $\frac{1}{2}a11^2 * i1$ for DZ twin pairs (as MZ twin share 100% of their segregating genes whereas DZ twins share half). However, under the PH causes PD hypothesis (Figure 2b), the cross-twin cross trait correlation can only come about through the variance of PH, therefore being $C22^2 * i2$ for both MZ and DZ twin pairs (as they are not assumed to differ in their shared environment).
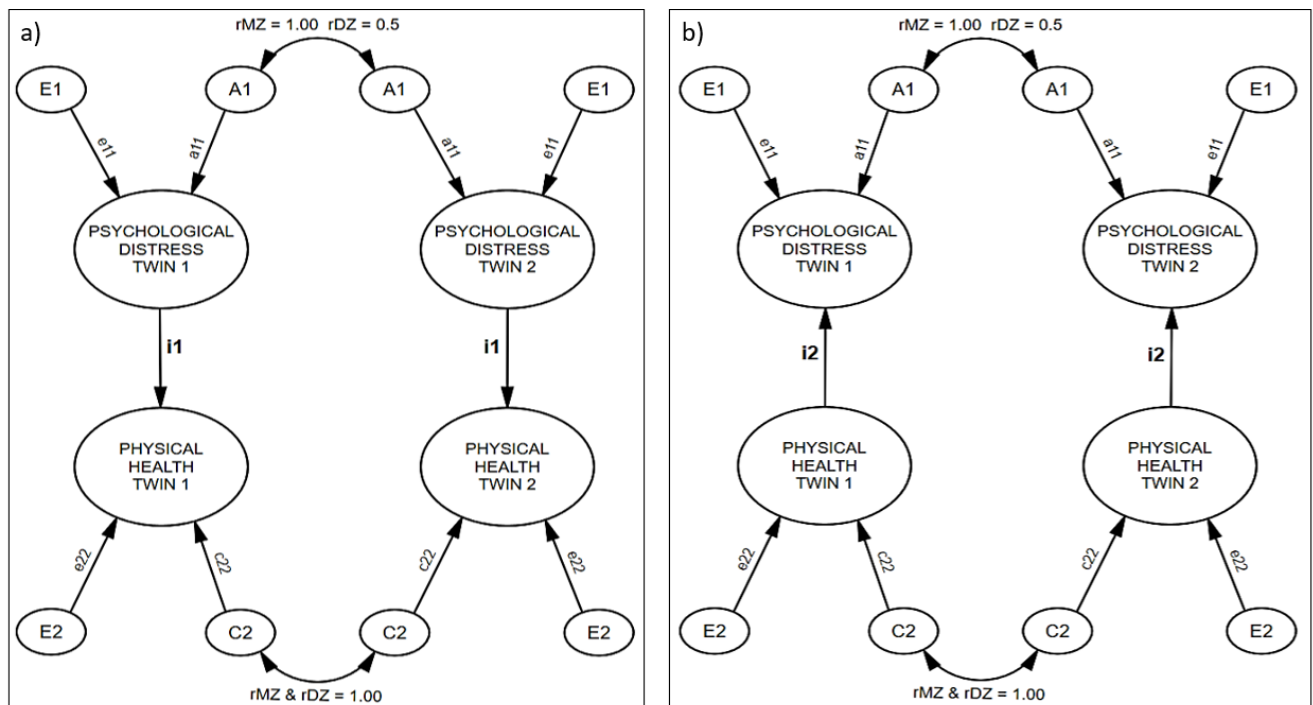

Figure 2. Direction of Causation twin model between psychological distress (PD) & physical health (PH). Figure 2a shows model with the likely direction of effect being PD → PH, whereby

the cross-twin cross-trait correlation (PD twin 1 – PH twin 2) will be $a11^2 * i1$ for MZ and $\frac{1}{2} a11^2 *i1$ for DZ twin pairs. Figure 2b depicts a model with the causal direction going from PH → PD, resulting in the cross-twin cross-trait correlation being $c22^2 * i2$ for both MZ and DZ twin pairs.

The power to detect DOC is therefore greatest when traits have very distinct aetiologies, giving differential cross-twin, cross-trait correlations. It is also possible to investigate DOC when traits have similar aetiologies but with differing proportions (e.g. one trait shows a higher genetic variance component). In essence, the familial influences A and C in a DOC model can be seen as instrumental variables, predicting a trait only through another intermediate trait. This is similar to the use of specific genetic variants or polygenic scores as instrumental variables in mendelian randomisation (Davey Smith & Hemani, 2014).

4. Specific ACE estimates for variables and factor loadings from the bivariate ACE model (95% CI)

Variable	A	C	E	Factor Loadings
ANXIETY	.00 (.00 / .03)	.00 (.00 / .05)	**.14 (.11 / .17)**	**.79 (.77 / .81)**
DEPRESSION	.01 (.00 / .05)	.06 (.00 / .02)	**.14 (.11 / .17)**	**.78 (.76 / .80)**
SOMATIC SYMPTOMS	**.22 (.08 / .29)**	.21 (.00 / .52)	**.35 (.24 / .54)**	**.37 (.34 / .41)**
EMOTIONAL WELLBEING	.01 (.00 / .11)	.04 (.00 / .09)	**.34 (.29 / .39)**	**.68 (.65 / .70)**
ROLE EMOTIONAL	.01 (.00 / .05)	.00 (.00 / .02)	**.26 (.21 / .31)**	**.58 (.55 / .61)**
GENERAL HEALTH	.02 (.00 / .14)	.07 (.00 / .16)	**.54 (.47 / .60)**	**.43 (.40 / .47)**
PHYSICAL FUNCTIONING	.04 (.00 / .19)	.04 (.00 / .14)	**.53 (.43 / .59)**	**.44 (.40 / .47)**
ROLE PHYSICAL	.03 (.00 / .18)	.01 (.00 / .02)	**.31 (.22 / .36)**	**.76 (.73 / .78)**
PAIN	.05 (.00 / .19)	**.28 (.19 / .32)**	**.13 (.10 / .16)**	**.82 (.79 / .84)**

5. Specific ACE estimates for variables from the unidirectional causation model (95% CI)

Variable	A	C	E
ANXIETY	.00 (.00 / .03)	.00 (.00 / .04)	**.14 (.11 / .17)**
DEPRESSION	.01 (.00 / .05)	.00 (.00 / .02)	**.14 (.11 / .17)**
SOMATIC SYMPTOMS	**.22 (.08 / .29)**	.21 (.00 / .52)	**.35 (.24 / .54)**
EMOTIONAL WELLBEING	.01 (.00 / .11)	.04 (.00 / .09)	**.34 (.29 / .39)**
ROLE EMOTIONAL	.01 (.00 / .05)	.00 (.00 / .02)	**.26 (.21 / .31)**
GENERAL	.02 (.00 / .16)	.08 (.00 / .17)	**.60 (.53 / .66)**

HEALTH			
PHYSICAL FUNCTIONING	.05 (.00 / .21)	.05 (.00 / .16)	**.59 (.49 / .66)**
ROLE PHYSICAL	.03 (.00 / .18)	.01 (.00 / .03)	**.39 (.26 / .44)**
PAIN	.06 (.00 / .23)	**.33 (.22 / .38)**	.15 (.11 / .19)

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Summary of chapter 5

Chapter 5 used the direction-of-causation (DOC) twin design to explore likely causal pathways between latent psychological distress and physical health factors. We find tentative support in favour of a unidirectional model running from psychological distress to physical health, although no DOC model could be confidently selected over the other. In the next chapter, we zone into psychological distress further, and given that the previous empirical chapters focused on a behaviour genetic perspective, chapter 6 provides an epigenetic angle to the thesis. The next chapter offers a novel viewpoint into the relationship between global DNA methylation and psychological distress in a sample of young adolescents.

Chapter 6 . Exploring Associations Between Global LINE-1 DNA Methylation And Psychological Distress Amongst Young Adolescents

Supplementary material can be found at the end of the chapter.

Exploring Associations Between Global LINE-1 DNA Methylation And Psychological Distress Amongst Young Adolescents

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Abstract

Objectives

Symptoms of psychological distress (including anxiety and depression) are common and known to have genetic underpinnings. There is, however, less known about the epigenetic contributions to this mental health domain especially in the context of global DNA methylation amongst young adolescents.

Methods

In this study, we explored relationships between measures of psychological distress (Mood's & Feeling's Questionnaire and the Strengths & Difficulties Questionnaire, both in combination and separately) and global DNA Methylation measured via the Long Interspersed Nuclear Element-1 (LINE-1), a repetitive element in the genome routinely used as a robust surrogate biomarker for global DNA methylation levels. Young adolescents (N=155, mean age 11.94 ±1.32) were recruited from the UK Twin's Early Development Study (TEDS) and the Social Relationships Study (SRS).

Results

Psychological distress, quantified using MFQ and SDQ questionnaires together and separately, did not significantly predict variance in global DNA methylation in our early adolescence blood nor buccal DNA samples.

Conclusions

Our study, to our knowledge, is the first to interrogate the relationship between both broad and specific quantitative measures of psychological distress with LINE-1 global DNA

methylation in a young adolescent cohort. Further research in larger, more diverse samples is required to better understand the role of global DNA methylation in relation to psychological distress.

Introduction

Psychological distress, as defined by anxiety and depression, is a complex, heterogeneous, and highly prevalent trait in the population (Drapeau et al., 2012; Wittchen et al., 2011). It is now understood that anxiety and depression are dimensional and normally distributed, with a range of factors contributing to their development (Meier & Deckert, 2019; Mullins & Lewis, 2017; Rijsdijk et al., 2003; Smoller, 2016). Previous twin studies have indicated moderate heritability for anxiety and depression related traits (Flint & Kendler, 2014; Smoller, 2016) and recent molecular genetic evidence has begun to indicate the role of common genetic variants (Howard et al., 2019; Levey et al., 2020, 2021; Purves et al., 2020). Twin studies also suggest the importance of adolescence as an emerging developmental period for adulthood psychopathology, with genetic and environmental continuity and innovation reported (Waszczuk et al., 2014; Zavos et al., 2012). There is, however, relatively limited epigenetic research in this area. This carries importance, considering that genetic studies do not explain all phenotypic variance (suggesting environmental influence/ gene-environment interplay) and discordance among monozygotic (identical) twins suggests the role of mechanisms other than genes. DNA methylation, an epigenetic mechanism, is one way that gene expression can change without altering the underlying DNA sequence (Schübeler, 2015).

Previous studies in this area focus on clinical presentation of psychological distress, with

differential DNA methylation patterns observed in different anxiety and depressive disorders (Czarny et al., 2021; Dempster et al., 2014; Schiele & Domschke, 2018; Shimada-Sugimoto et al., 2017), across trajectories (Bortoluzzi et al., 2018; Perna et al., 2020) and in several candidate genes (Bartlett et al., 2017; Gottschalk & Domschke, 2016; Lin & Tsai, 2019; Roberts et al., 2015, 2019). There is, however, limited evidence for genes or genomic regions consistently associated with these disorders and research conducted to date has so far provided limited information on the dimensional nature of anxiety or the global influence of the epigenome.

Commonly used approaches such as epigenome-wide association studies provided novel discoveries of several differentially methylated sites and regions (significantly) associated with anxiety and depressive traits (Alisch et al., 2017; Shimada et al., 2018; Starnawska et al., 2019). However, gene and region-specific DNA methylation still does not provide sufficient information on the globality of the epigenome. As an alternative approach, global DNA methylation has been gauged using repetitive elements in DNA such as short and long interspersed nuclear elements (LINEs and SINEs). LINE-1 is the largest member of the LINE family with over 500,000 copies, estimated to comprise approximately 17% of the genome (Lander et al., 2001). DNA methylation levels at LINE-1 sequences are highly correlated with global DNA methylation levels (Weisenberger et al., 2005) and provides a comprehensive surrogate approach for global genomic DNA methylation (Müller et al., 2021). Global LINE-1 DNA methylation has been previously investigated in neurodevelopmental conditions including autism spectrum disorder (Tangsuwansri et al., 2018) as well as schizophrenia and bipolar disorder (Li et al., 2018; Murata et al., 2020).

Anxiety and depression have typically been referred to as stress-related disorders, and stress has been shown to associate with global DNA methylation levels (Bakusic et al., 2017). One such study finds that chronic stress is correlated with increased global DNA methylation (Duman & Canli, 2015). Two previous studies also reported global DNA hypermethylation in anxious and depressed individuals (N = 25 and 38) in comparison to controls (N = 22 and 78) with higher levels of global DNA methylation significantly associated with anxiety/depression severity (Murphy et al., 2015; Reszka et al., 2021).

One other study, however, reported LINE-1 hypomethylation in individuals with major depressive disorder (N=105) (Liu et al., 2016) and another observed an inverse correlation between depression scores and global DNA methylation levels in depressive disorder patients (N=49) (Tseng et al., 2014). This is reiterated in a discordant twin sample (N=24), whereby depression was associated with decreased global DNA methylation in female discordant pairs in comparison to controls (Byrne et al., 2013). One other study reports no significant differences in global DNA methylation levels (as quantified by the LINE-1 marker at four sites) between depression patients and controls (Nantharat et al., 2015). Given these conflicting findings and limited sample sizes comprising of adults, further research is required to better understand the relationship between global DNA methylation and psychological distress, especially at younger age groups.

Young adolescence is an important period of development for long-term mental health, with anxiety and depressive disorders often emerging between 11-18 years of age (Maughan et al., 2013; Patton et al., 2014). Yet, work on global DNA methylation thus far focus primarily on adult samples. This developmental window can therefore be a novel area to identify relevant

biomarkers for psychological distress, including DNA methylation, early on in the life course. In addition, previous work mainly adopts case-control designs, focusing on the clinical presentation of anxiety and depression. Yet, there is increasing evidence for the dimensional, continuous nature of psychopathology (Krueger et al., 2018). In this explorative study, we investigate psychological distress using two different quantitative measures, the Mood's & Feeling's Questionnaire (MFQ) and the Strength's and Difficulties Questionnaire (SDQ). As well as analysing these measures separately, the two measures were combined into a total psychological distress score, in line with anxiety and depression loading onto a general internalising/ psychopathology 'p' factor (Allegrini et al., 2020; Caspi et al., 2014). Hence, we explored associations between global LINE-1 DNA methylation profiles from blood and buccal samples with 1) MFQ and SDQ scores and 2) a total composite psychological distress score in a sample of young adolescents.

Methods

Participants were recruited from the UK Twins Early Development Study (TEDS) (Rimfeld et al., 2019) and UK Social Relationships Study (SRS), a sub-study of TEDS. The sample consisted of a total of 155 participants, including 73 monozygotic (identical) complete twin pairs (N =146) and 9 incomplete monozygotic twin individuals. The sample was originally recruited as part of an autism epigenetics study (Wong et al., 2014), and therefore also includes individuals scoring high on the childhood autism spectrum test (CAST) (Scott et al., 2002). The mean age for the sample was 11.94 years (SD = 1.32) and most of the sample comprised of males (61%).

Psychological distress was measured using two separate scales, derived from both parent and child reports. First, a total score from the Mood's and Feeling's Questionnaire (MFQ) (Angold et al., 1995) was calculated from 11 items including 'I didn't enjoy anything at all' and 'I felt lonely' with participants indicating how they felt about each item on a Likert scale ranging from 'Not true' (scored 0), 'Quite true' (1) and 'Very true' (2). Second, we included a total score from the five-item emotional problems sub-section of the Strengths and Difficulties Questionnaire (SDQ) (Goodman, 1997). Participants rated how they felt on items such as 'I worry a lot' and 'I am often unhappy, downhearted or tearful' on a Likert scale ranging 'Not true' (scored 0), 'Quite true' (1) and 'Very true' (2).

Trained phlebotomists extracted whole-blood samples at age 15 (TEDS) and buccal samples were collected using cheek swab kits provided to participants at the ages of 13 (SRS) and 15 (TEDS). DNA was extracted from a total of 227 samples, including blood (N=103) and buccal (N=124) samples from a total of 155 participants, using standard procedures (Freeman et al., 2003; Wong et al., 2014).

Global LINE-1 DNA methylation analysis

We quantified DNA methylation at LINE-1 (Long Interspersed Nuclear element-1) repetitive elements to gauge global DNA methylation, with further experimental procedure detailed in ***Supplementary material 1***. Briefly, genomic DNA (500 ng) for each individual was treated with sodium bisulphite using the EZ-96 DNA Methylation-Gold ™ kit (Zymo Research, CA, USA) following the manufacturer's protocol. Bisulfite treated DNA was amplified using polymerase chain reaction (PCR) with primers designed based on previous literature

(Guarrera et al., 2015; Wang et al., 2010; Yang et al., 2004). The Agena Bioscience EpiTYPER MassARRAY® platform was used to quantify DNA methylation levels (Suchiman et al., 2015), and an average measure of global DNA methylation based on six CpG sites was obtained. Stringent quality control steps were implemented and detailed in *Supplementary material 2*.

Statistical analysis

We conducted two lines of analysis in the current study. First, we conducted analyses on MFQ and SDQ total scores separately. Distributions of the MFQ and SDQ scores and LINE-1 DNA methylation in blood and buccal samples are illustrated in *Supplementary material 3*. Second, we performed Z-score transformation on both MFQ and SDQ and then combined the two scales into a total psychological distress composite score. Distributions of total psychological distress scores following z-transformation is displayed in *Supplementary material 4.*

We conducted mixed multiple regression analyses with parent and child total psychological distress scores regressed onto LINE-1 DNA methylation scores in blood and buccal separately. We also included age, male gender, family ID and childhood autism spectrum test (CAST) scores as covariates. The latter is included due to the nature of the sample, selected originally for an autism epigenetics study (Wong et al., 2014). For the buccal data analysis, cohort was also included as a covariate as the sample set comprised of participants recruited from two different cohorts, namely TEDS and SRS. Both cohort and family ID effect were entered as random variables. Given the tissue-specific nature of the epigenome

(**_Supplementary material 3_**), parallel analyses were conducted for global LINE-1 DNA methylation profiled from blood and buccal samples. All analyses were conducted in R with mixed multiple regression output obtained using the 'lme4' (Bates et al., 2018) and R squared (R^2) results obtained using the 'MuMIn' package (Barton & Barton, 2015). Significance of statistical tests were compared to the Bonferroni-adjusted p-value threshold (.05 / 12 tests = 0.004).

We also conducted within-twin analyses on the 73 monozygotic twin pairs available (N=146). The MZ twin differences design correlates differences between twin 1-2 on a trait of interest (e.g., psychological distress) with differences between twin 1-2 on another trait (e.g., Global LINE-1 DNA methylation). As MZ twins are genetically identical and not assumed to differ in terms of their shared environment, familial effects are controlled for, making the subsequent association truly environmental. This may further inform on the epigenetic nature of anxiety.

Results

Sample details are outlined in **Table 6.1** with descriptive statistics on the variables used summarised in **Table 6.2**.

Table 6.1. Sample characteristics & descriptive statistics

Study	Mean Age (SD)	MZ Complete Twin pairs (individuals)	MZ Incomplete twins (individuals)	Total participants	Tissue	Total DNA records	Sex M:F
SRS	13.18 (1.17)	22 (N = 44)	8	52	52, Buccal	52	39:13
TEDS	11.16 (.62)	51 (N=102)	1	103	72, blood + buccal data N = 144 samples 31, blood data only	175	56:47
Total	11.94 (1.32)	73 (N = 146)	9	155		227	95:60

SRS = Social Relationships Study; TEDS = Twin's Early development Study; MZ = Monozygotic; M = Male; F = Female.

Table 6.2. Means, SDs and maximum ranges for variables.

Variable	Mean	SD	Max possible range
Parent MFQ	.82	1.89	0-22
Parent SDQ	1.84	1.99	0-10
Child MFQ	1.92	2.49	0-22
Child SDQ	2.11	1.93	0-10
LINE-1 DNA Methylation, Blood	68.78	1.49	0-100
LINE-1 DNA Methylation, Buccal	49.53	4.71	0-100

MFQ = Mood's & Feeling's Questionnaire; SDQ = Strength's & Difficulties Questionnaire. LINE-1 = Long Interspersed Nuclear Element – 1.

Findings from the mixed multiple regression analyses for the MFQ and SDQ total scores are presented in **Table 6.3**. Overall, we did not find a significant contribution of parent and child reported MFQ and SDQ scores to variance in blood or buccal LINE-1 DNA methylation. The effect of male gender was significant in predicting LINE-1 DNA methylation in blood analyses. Scatterplots showing Pearson's correlations for these variables are displayed in *Supplementary material 5*.

Table 6.3. Mixed multiple regression results with child and parent reported MFQ and SDQ total scores and LINE-1 DNA methylation in blood and buccal samples.

Psychological distress variable	Reporter	Tissue	Adjusted Coefficient (SE)	Unadjusted coefficient (SE)	t	p	95% CI	R^2, marginal (conditional)
MFQ	**Parent**	Blood	-.05 (.10)	.01 (.09)	-.50	.62	-.24 / .14	.14 (.15)
	Child		-.01 (.07)	.00 (.07)	-.11	.91	-.13 / .12	.14 (.16)
	Parent	Buccal	.07 (.28)	.10 (.24)	.25	.81	-.46 / .60	.01 (.43)
	Child		-.14 (.22)	-.09 (.20)	-.65	.52	-.57 / .29	.01 (.43)
SDQ	**Parent**	Blood	.07 (.09)	.04 (.08)	.82	.42	.10 / .24	.15 (.16)
	Child		.07 (.10)	.02 (.10)	.67	.51	-.12 / .25	.15 (.20)
	Parent	Buccal	.42 (.29)	.41 (.25)	1.45	.15	-.13 / .98	.06 (.40)
	Child		.16 (.27)	.11 (.23)	.59	.56	-.37 / .66	.01 (.43)

Adjusted models controlling for age, gender, CAST (Childhood Autism Spectrum Test) scores. Buccal analyses are additionally adjusted for cohort effect. The t scores, p-value and R^2 refer to the adjusted models. Marginal $R^2 = R^2$ due to fixed effects, Conditional $= R^2$ due to full model.

To further explore general psychological distress and in line with research supporting the

loading of anxiety and depression under an umbrella psychopathology factor, we conducted

parallel mixed regression analyses using the composite psychological distress score

combining the MFQ and SDQ scales. Parent or child reported total psychological distress

scores did not significantly predicted LINE-1 DNA methylation **(Table 6.4)**. Pearson's

correlation scatterplots of parent and child reported psychological distress and LINE-1 DNA

methylation is displayed in **Figure 6.1**.

Table 6.4. Mixed multiple regression results with child and parent reported psychological
distress total scores and LINE-1 DNA methylation in blood and buccal samples.

Psychological distress variable	Tissue	Reporter	Adjusted Coefficient (SE)	Unadjusted coefficient (SE)	t	p	95%CI	R^2, marginal (conditional)
Total score	Blood	Parent	.03 (.12)	.04 (.10)	.25	.81	-.20 / .26	.14 (.17)
		Child	.04 (.12)	-.02 (.11)	.37	.72	-.18 / .26	.15 (.19)
	Buccal	Parent	.27 (.34)	.33 (.29)	.79	.43	-.38 / .93	.04 (.41)
		Child	.00 (.33)	.01 (.29)	-.01	.99	-.64 / .63	.01 (.42)

Adjusted models controlling for age, gender, CAST (Childhood Autism Spectrum Test) scores.
Buccal analyses are additionally adjusted for cohort effect. The t scores, p-value and R2 refer to the
adjusted models. Marginal R^2 = R^2 due to fixed effects, Conditional = R^2 due to full model.

Figure 6.1. Pearson's Correlations between total psychological distress scores from parents and children and LINE-1 DNA Methylation in blood and buccal samples
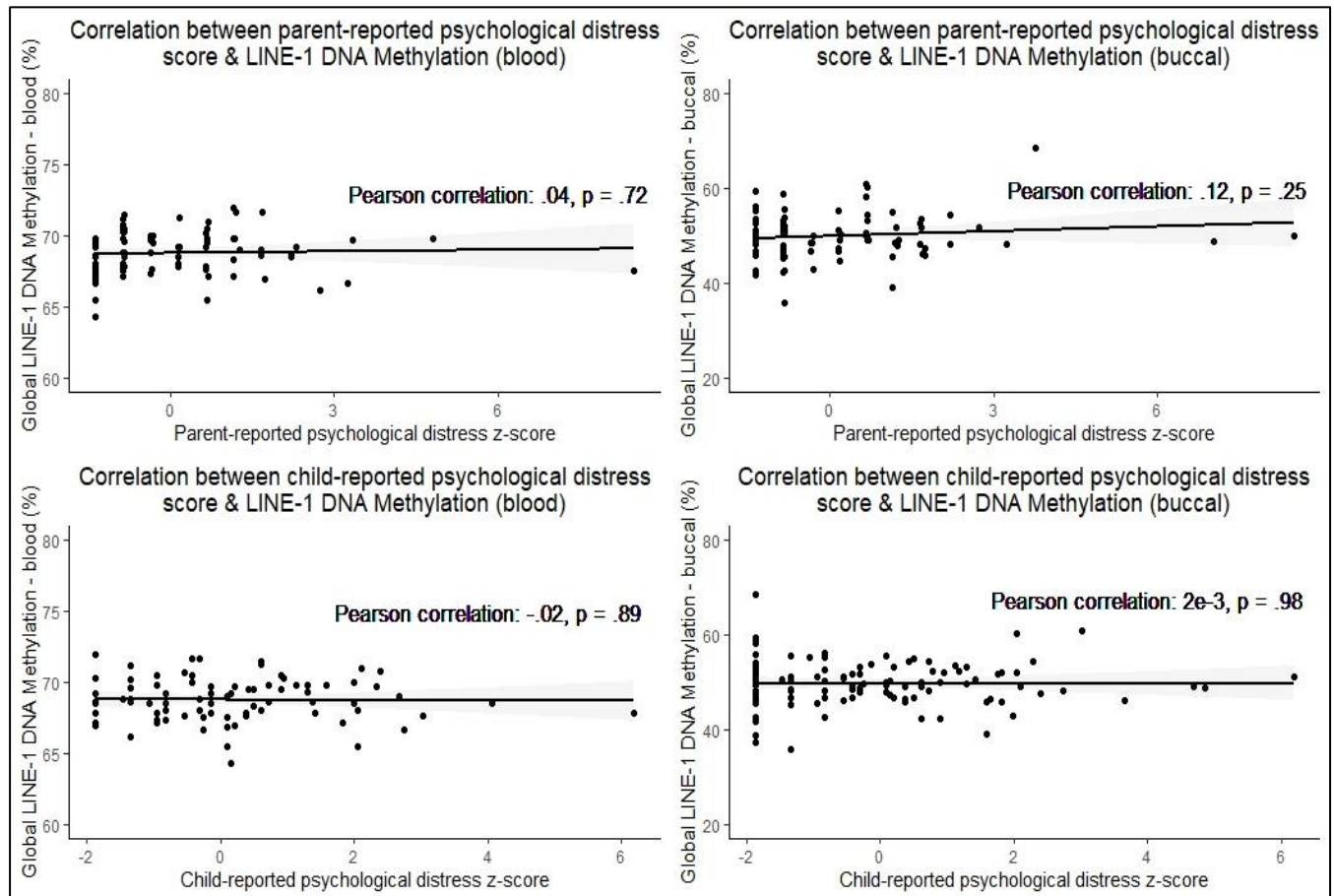
Within-twin analyses were also conducted in the form of the MZ twin difference design. This correlates differences in one variable (e.g., Differences in total psychological distress scores) with another (e.g., Differences in blood DNA methylation). To account for ASD status in the sample, we conducted these in the known Autism Spectrum Disorder (ASD) groupings. As expected, these within twin analyses mirrored the phenotypic relationships, with no significant correlations. More details on the twin status of the sample are detailed in **Table 6.5** and **Table 6.6** depicts these within-twin analyses.

Table 6.5. Twin and singleton status of sample, split by ASD grouping.

Concordance status	Total				Blood	Buccal
	No. of DNA Records[1]	Twin Pairs[1]	Twin Individuals[1]	Singletons	Twin Pairs (Individuals)	Twin Pairs (Individuals)
Concordant Control	72	24	48	6	10 (20)	23 (46)
Concordant for ASD	35	13	26	3	6 (12)	10 (20)
Discordant for ASD	17	6	12	1	5 (10)	3 (6)
Discordant for communication	34	10	20	0	10 (20)	7 (14)
Discordant for social	36	10	20	0	10 (20)	8 (16)
Discordant for RRBIs	33	10	20	1	10 (20)	6 (12)
Total	227	73	146	11	51 (102)	57 (114)

[1] Note that the number of DNA records, total twin pairs and total twin individuals include participants with both blood and buccal data.

Table 6.6. MZ Within-twin pair difference analyses in the different Autism Spectrum disorder (ASD) groups.

Concordance status	Parent-report total score		Child-report total score	
	Blood	Buccal	Blood	Buccal
Concordant Control	r = -.20, p=.63	r = .09, p=.72	r = -.42, p =.35	r = -.02, p=.95
Concordant for ASD	r = .003, p =.99	r = .21, p =.69	r = -.77, p=.23	r = .30, p=.43
Discordant for ASD	Not enough observations (NAs >3)	Not enough observations (NAs >3)	Not enough observations (NAs >3)	Not enough observations (NAs >3)
Discordant for communication difficulties	r = .01, p =.98	r = .51, p =.29	r = -.001, p =.99	r = .35, p=.50
Discordant for social difficulties	r = -.21, p=.58	r = -.23, p=.63	r = -.12, p=.78	r =.42, p=.34
Discordant for RRBIs (Restricted, repetitive behaviours/ interests)	r = .27, p=.48	r = -.69, p=.20	r =.37, p=.32	r = -.55, p=.33
Discordance overall[1]	r = .02, p = .93	r = .05, p =.85	r = .17, p = .39	r = .01, p =.96

Note: [1]Discordance overall group refers to all those who are discordant for both ASD diagnosis and the triad of impairments symptoms.

Discussion

In this exploratory study, we find that psychological distress, as reported by both children and parents, did not predict variance in global LINE-1 DNA methylation. Within-twin analyses also mirrored these findings. This study, to our knowledge, is one of the first to investigating the role of global LINE-1 DNA methylation in the context of psychological distress in a young adolescent sample and thus provides a novel contribution and merits further discussion.

Strengths

To date, there has been limited work on the epigenetic nature of psychological distress (state, trait, or disorders) in the framework of global DNA methylation. Most work using the LINE-1 repetitive element as a global DNA methylation marker has focused on disorders such as schizophrenia and bipolar disorder, with limited work on the internalising problems spectrum. A previous study indicated global DNA hypermethylation in anxious individuals (n= 25) in comparison to controls (n=22), with global DNA methylation significantly associated with anxiety (Murphy et al., 2015). Another comparison study with a female only sample set also reported LINE-1 DNA hypermethylation in clinically depressed individuals (N=38) compared to controls (N=78) (Reszka et al., 2021). Both studies suggested that LINE-1 DNA methylation was associated with anxiety and depression severity. These previous studies, however, adopt a case-control design, are different in terms of sample characteristics, quantification of global DNA methylation, as well as study aims, possibly leading to differing results. In this study, our aims were purely exploratory with no a priori expectations for results. Additionally, most previous studies focus either on anxiety or depression whereas we

not only analysed the MFQ and SDQ scales separately, but also adopted a combined measure of psychological distress to gauge a more comprehensive perspective on the internalising problems spectrum. As well as the largest sample size of this study kind, our study is a first considering a young adolescent sample and adopts a dimensional view on psychological distress, in line with research supporting psychopathology as a continuum rather than categories (Krueger et al., 2018).

Limitations and future directions

The major limitation of the current study is the sample size, although being the largest to date (Murphy et al., 2015; Reszka et al., 2021), remains small-scale. In addition, the sample also includes a small proportion of individuals scoring high on autism trait severity which, although controlled for partly in the analyses, could introduce bias in the results. Furthermore, although the quantitative measure of psychological distress is in line with efforts to capture the dimensionality, the sample scores relatively low on psychological distress (< 2 for MFQ and <3 for SDQ scores out of a possible 22 or 10, respectively). It may therefore be worth extending this work to other sample sets to introduce more variability and capture the full spectrum of emotional problems.

Additionally, we defined global DNA methylation based on only one repetitive marker, quantified through 6 CpG sites. The method has gained attention in recent years and could be a valuable biomarker for psychological health (Misiak et al., 2019). It is albeit worth extending this work to other methods such as quantifying DNA methylation through the Alu repetitive element, as done by others (Li et al., 2019; Reszka et al., 2021). We did not find significant relationships between LINE-1 DNA methylation and measures of psychological distress here, and a previous study also indicates that depression symptomatology was

associated with methylomic changes at the gene-level rather than at a global level (Starnawska et al., 2019). The role of global DNA methylation in relation to psychological distress therefore requires further exploration. Future work may benefit from collaborative efforts such as the inclusion of epigenetic research within the Psychiatric Genomics Consortia (PGC) for anxiety and depression (Meier & Deckert, 2019).

In conclusion, we did not find that psychological distress, reported by both parents and children, significantly predicted variance in LINE-1 DNA methylation in blood or buccal profiles, in our adolescent sample. Future work using this relatively novel method on larger, more heterogeneous samples may further explore the relationships with psychological distress and extrapolate results more comprehensively. Although preliminary, our exploratory work provides new insights and provides a basis for future studies in this arena.

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Supplementary material for Chapter 6

Supplementary Material 1. **Global LINE-1 DNA methylation analysis experiment details**

Primer design

Forward and reverse primers were designed using EpiDesigner
(https://www.epidesigner.com/) and BiSearch (http://bisearch.enzim.hu/). Primer sequence
and CpG site coverage were validated using the RSeqMeth package in R (https://cran.r-
project.org/src/contrib/Archive/RSeqMeth/). This ensures that the target sequence covers the
CpG sites of interest and are not overlapping with other CpG sites.

Name of primer	*Use*	*Forward primer*	*Reverse primer*
LINE-1	Estimating Global DNA methylation	aggaagagagGTGTGAGGTGTTAGTGTGTTTTGTT	cagtaatacgactcactatagggagaaggctATATCCCACACCTAACTCAAAAAAT

Bisulfite conversion protocol

We followed the manufacturer's protocol for EZ-96 DNA Methylation-Gold ™ Kit (Zymo
Research, CA, USA) (https://files.zymoresearch.com/protocols/_d5007_ez-
96_dna_methylation-gold_kit.pdf) with minor adjustments to 'Step 9' :

1 - Add 25μl of M-Elution Buffer to each well. Stand for 2 minutes. Centrifuge at 4000g for 3
minutes

2 - Add 20μl of M-Elution Buffer to each well. Stand for 2 minutes. Centrifuge at 4000g for 3
minutes

3 - Add 45μl of solution in collection plate to each well. Stand for 2 minutes then centrifuge
at 4000g for 3 minutes.

LINE-1 Global methylation PCR mix

Reagent	*Volume, 1 Reaction (μl)*
10X Buffer	1
$MgCl_2$	0.2
dNTPs mix (25 mM each)	0.08
F primer	0.4
R primer	0.4
Hot Start Taq polymerase (5U/μl)	0.08
H_2O	5.84
DNA	2
TOTAL	10

Thermal cycler steps

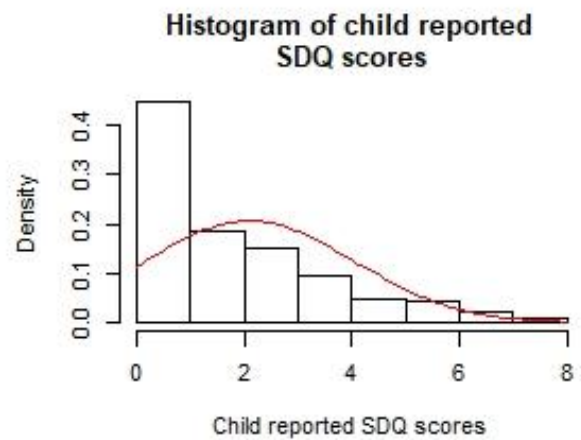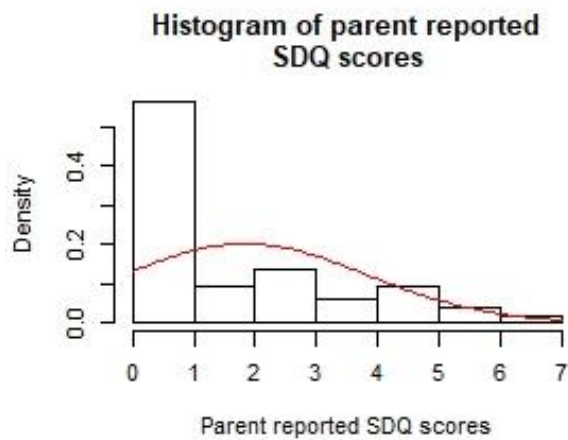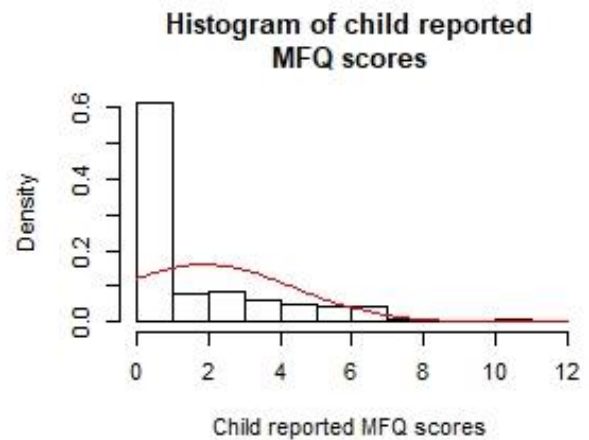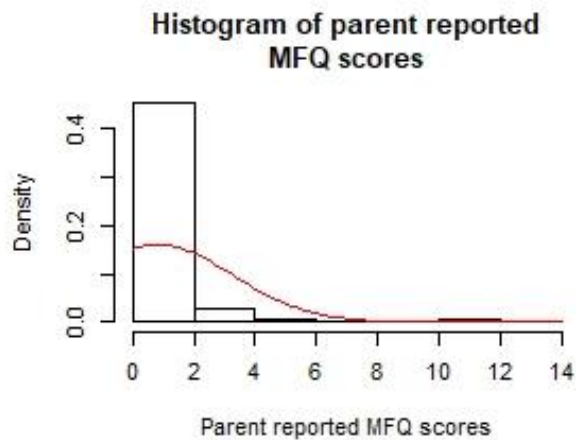| Step	Temperature (°C)	Duration (mins)
Step 1	94	4'
Step 2	94	20"
Step 3	56	30"
Step 4	72	1'
Step 5	Step 2, 44 times	
Step 6	72	3'
Step 7	4	10'
		TOTAL RUNNING TIME ~ 2hrs 24 mins

Supplementary Material 2. Quality control (QC) steps implemented

All data processing and statistical analyses were completed within the R statistical analysis environment (http://www.r-project.org). We confirmed that the CpG sites of interest were covered using Epidesigner, BiSearch and the RSeqMeth package in R.

Data quality control procedures:

- CpG site(s) that had >15% missing data were removed- 2 CpG sites.
- CpG site(s) that did not perform well for positive control sample, i.e., fully methylated control (Zymo research, CA, USA), were removed- 1 CpG site
- CpG site(s) that have <5% average methylation was removed- 0 CpG site
- In total, 6 (out of 9) CpG sites passed our stringent quality control steps, and an average DNA methylation value was calculated and used as an estimate of global DNA methylation.

Supplementary Material 3. Distributions of MFQ and SDQ scores and LINE-1 DNA Methylation in blood & buccal samples

Histogram of parent reported MFQ scores

Histogram of child reported MFQ scores

Histogram of parent reported SDQ scores

Histogram of child reported SDQ scores

Histogram of LINE-1 DNA methylation in blood samples

Histogram of LINE-1 DNA methylation in buccal samples
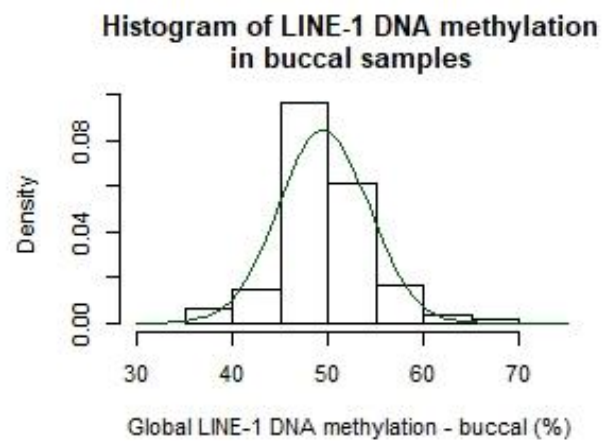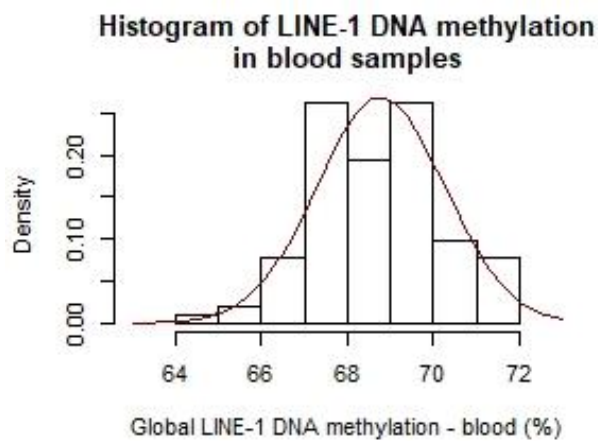
Supplementary Material 4. Distributions of z-transformed total psychological distress scores from parents and children.
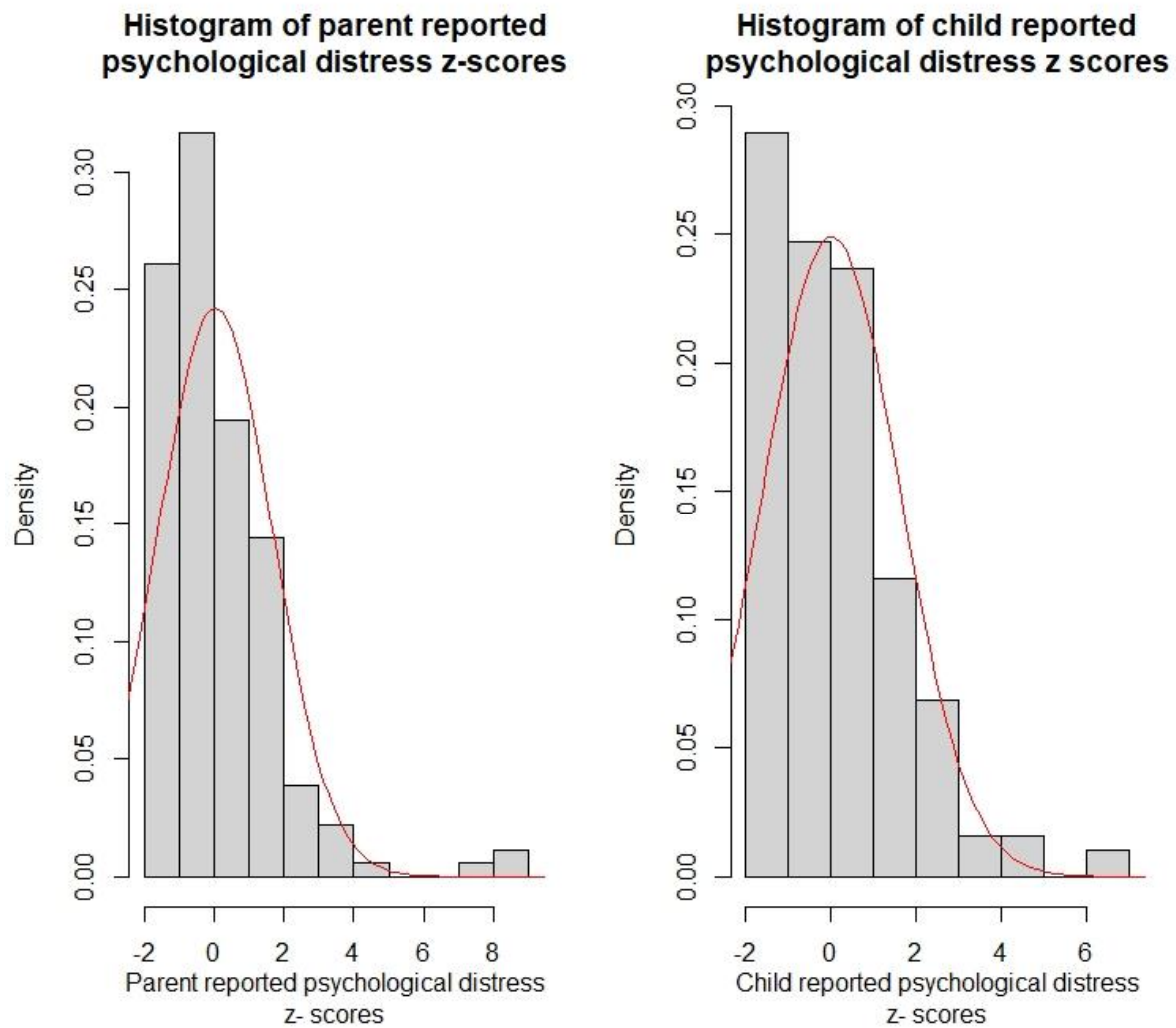
Supplementary Material 5. Correlations between parent & child reported MFQ & SDQ scores with LINE-1 DNA Methylation in blood and buccal samples.
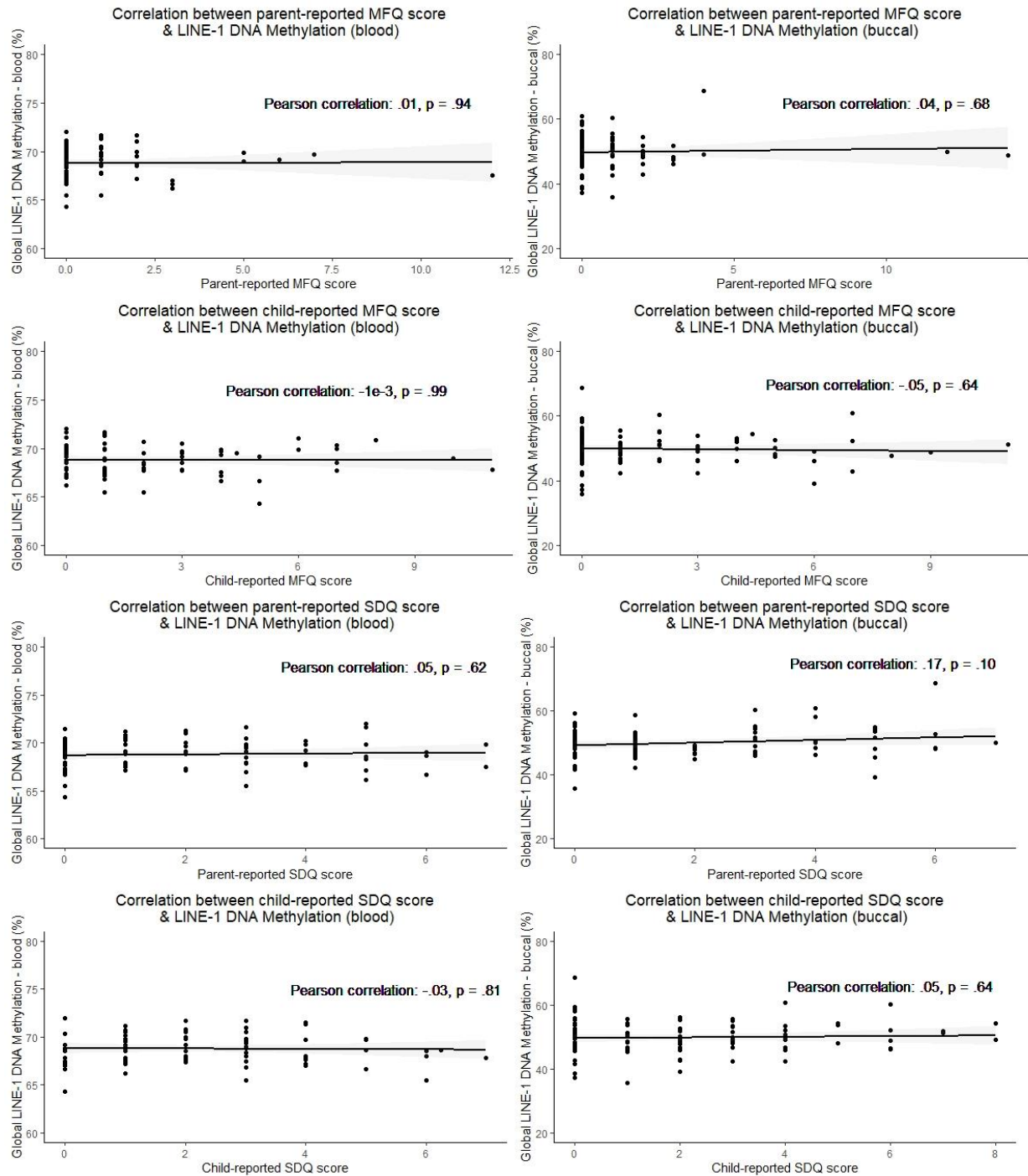
Supplementary Material 6. The 11-item Mood's & Feeling's Questionnaire (MFQ) and the 5-item emotional problems scale from the Strengths & Difficulties Questionnaire (SDQ).

These questions are about how you might have been feeling or acting recently. For each question, please tick the box you think shows how much you have felt or acted in this way in the past two weeks.

	Not true	Quite true	Very true
I didn't enjoy anything at all	☐	☐	☐
I felt so tired I just sat around and did nothing	☐	☐	☐
I felt I was no good anymore	☐	☐	☐
I cried a lot	☐	☐	☐
I found it hard to think properly or concentrate	☐	☐	☐
I hated myself	☐	☐	☐
I was a bad person	☐	☐	☐
I felt lonely	☐	☐	☐
I thought nobody really loved me	☐	☐	☐
I thought I could never be as good as other kids	☐	☐	☐
I did everything wrong	☐	☐	☐

Please give your answers on the basis of how things have been for you over the last 3 months.

	Not true	Quite true	Very true
I get a lot of headaches, stomach aches or sickness	☐	☐	☐
I worry a lot	☐	☐	☐
I am often unhappy, downhearted, or tearful	☐	☐	☐
I am nervous in new situations. I easily lose confidence	☐	☐	☐
I have many fears I am easily scared	☐	☐	☐

Summary of chapter 6

This final empirical chapter offers an exploratory approach to the relationship between global DNA methylation (as gauged through the LINE-1 repetitive marker in DNA) and psychological distress in a sample of young adolescents. Both regression analyses and within-twin analyses did not yield any significant associations. In the next chapter, I discuss the empirical chapters in this thesis in light of their strengths, limitations and propose possible future directions.

Chapter 7 . General discussion

7.1. Overall findings

The overarching aim of this thesis was to explore the associations between measures of

psychological distress with physical health and health related quality of life. Although mostly

surrounding anxiety, this thesis also encapsulates measures of depression, emotional

problems, and somatic complaints as part of the psychological distress umbrella. Overall, the

empirical chapters using twin model fitting suggested a) decreased physical functioning in

terms of cardiovascular autonomic reactivity with increasing levels of anxiety (chapter 3); b)

negative associations between anxiety and health related quality of life with genetic and

environmental underpinnings of their aetiology and associations (chapter 4) and c) potential

causal mechanism running from psychological distress to physical health problems (chapter

5). As well as twin model fitting, this thesis also involved epigenetic analyses, allowing d) an

exploration of the relationship between global DNA methylation and psychological distress

(chapter 6).

7.2. Chapter-specific findings

To address chapter-specific findings, I refer to the original aims of this thesis. A summary of

chapter-specific study characteristics and key findings can be found in **Table 7.1**.

1) Is there an autonomic basis to anxiety?

This first empirical study (chapter 3) aimed to explore the relationship between

cardiovascular autonomic measures and anxiety symptoms. Using a genetically sensitive

multivariate twin design, I explored: (a) how anxiety symptoms correlate with three

cardiovascular autonomic measures: Inter beat-interval (IBI), Heart-rate variability (HRV)

and Baroreflex sensitivity (BRS). Phenotypically, anxiety was negatively correlated with the

three indicators of cardiovascular health, though only the association with baroreflex

sensitivity was found to be significant. Although previous research suggests associations

between anxiety phenotypes and cardiovascular health, there has been limited research

investigating the autonomic basis to this. This chapter adds to the literature suggesting

autonomic decline with higher levels of anxiety (Chalmers et al., 2014, 2016; Vinkers et al.,

2021; Virtanen et al., 2003).

One of the biggest strengths of this chapter is going beyond phenotypic associations, also

exploring (b) the extent to which individual differences in anxiety symptoms and

cardiovascular autonomic measures are determined by latent genetic and environmental

factors. Findings suggest moderate heritability estimates for anxiety and the three autonomic

measures, with the estimate for inter-beat interval being significant. This is the first

multivariate twin study investigating the association between anxiety and cardiovascular

autonomic measures, with a comparison study investigating neuroticism (Riese et al., 2007).

Yet, neuroticism is perhaps best identified as a personality trait common to mood and anxiety

disorders (Barlow et al., 2014; Griffith et al., 2010) and may not fully capture the anxiety

spectrum whereas several questionnaires specifically designed to measure anxiety were used

in chapter 3.

Finally, we investigated (c) the genetic and environmental underpinning of the associations

between the anxiety-cardiovascular domains. Results suggest non-significant aetiological correlations although the association between anxiety – BRS was mostly driven by common-environmental influences. Although previous research has implied negative associations, there has been no investigation to date offering a behaviour genetic perspective on these relationships. The twin design allowed exploration of this, although larger sample sizes may reduce the large confidence intervals surrounding these parameter estimates.

Intermediate phenotypes such as autonomic functioning and the HPA axis are an area of interest as a bridge between mental and physical health (Gottschalk & Domschke, 2017; Ottaviani et al., 2009; Vinkers et al., 2021). Ultimately, this chapter adds a genetically informative standpoint on the relationship between anxiety and cardiovascular autonomic functioning.

2) How does the relationship between mental-physical health manifest in a non-western setting?

Building on from chapter 3, Chapter 4 takes a broader approach to mental-physical health and capitalizes on the Sri Lankan population-based Colombo twin and singleton study (CoTASS). This dataset was firstly used to investigate a) the genetic and environmental variance components of anxiety symptoms and health related QoL. Results generally suggest low heritability, with only emotional wellbeing being significant. Interestingly, substantial influence of the common environment was found for several health related QoL domains. For

many twin studies, this shared environmental influence is largely non-significant and often dropped from twin modelling analyses. Yet, for this study and especially for women, the shard environmental component showed significant influence on the variance of anxiety and health related QoL. This indicates a possible cultural difference compared to western populations, whereby environmental influences explain more variance in these traits. This is also reiterated with a significant and large contribution of the unique environment (including measurement error) for all measures, suggesting the role of individual-specific environmental factors. We argue that increased environmental variability in Sri Lanka may have contributed to an attenuation of genetic effects and increased role of environmental effects.

This study also explored b) phenotypic correlations, with significant and negative associations found between anxiety and all eight measures of health-related QoL. Although anxiety is known to reduce quality of life and general functioning (Beard et al., 2010; Johansson et al., 2013), no study to date has combined all these measures in a comprehensive method. We also investigated c) the extent to which overlapping genetic and environmental factors underlie these associations and d) sex differences in these parameters. The phenotypic correlations' breakdown suggests a significant contribution of common environmental influences to these associations in women. In men, these associations were mostly explained by unique environmental effects. Bivariate genetic model fitting mirrors these results whereby significant common environmental correlations are reported for women but not men. This is contrary to western populations where shared environmental correlations are generally low or non-significant. We did not yield significant genetic correlations, contrasting what is known through molecular genetic studies, yet we employed a twin design and may have been

statistically underpowered to detect such effects. All unique environmental correlations were significant, again implying individual-specific factors that may increase anxiety and poor health-related QoL.

Given that most published research centers around European and more economically developed countries, this study is an important step to introduce more diversity in behaviour genetics research. This chapter suggests differences in variance components as well as in associations between anxiety and health related QoL pointing to socio-demographic and sex-specific factors.

3) What is the possible causal mechanism between psychological wellbeing and physical health (chapter 5)?

Although chapter 4 provides insight into the mental-physical health relationship, it remains correlational. The 5th empirical chapter in this thesis examines whether there is a causal direction in the relationship between physical and psychological health. Using cross-sectional, genetically informative data from the Sri Lankan population-based twin and singleton sample, this causal direction was tested between two latent factors: Psychological distress (explained by measures of Anxiety, Depression, Emotional wellbeing, and Somatic Distress) and Physical health (Measured by General health, physical functioning, energy/fatigue, and pain). We tested, in succession, using direction-of-causation twin models whether a) psychological distress causes decrease in physical health reports, or b) vice versa,

c) reciprocal causation or d) no causal links between the two factors. Findings suggest tentative support for a unidirectional causation model in which poor psychological distress may be a precursor of poor physical wellbeing. However, it must be noted that differences between model fit indices were relatively small, and the next most feasible model was the reciprocal model. Hence, although interesting, these results remain preliminary.

Nevertheless, this chapter provides novel insights into the notion of causality between mental-physical health. Through manipulation of the twin method, we show that causality can be disentangled using cross-sectional data. In addition, direction-of-causation twin models have thus far utilised western samples such as Australian and American populations (Gillespie et al., 2003, 2012), whereas here, for the first time, we applied this twin model to a non-western sample.

4) Is there an epigenetic basis underlying psychological distress?

Most of the empirical chapters in this thesis presents twin modelling analyses which focuses on disentangling variance of traits into latent genetic and environmental components. This final empirical chapter adds to research on non-genetic, epigenetic factors underlying the occurrence of psychological distress in the population. Chapter 6 aimed to explore associations between global DNA methylation and psychological distress, in particular amongst young adolescents. Previous studies have mostly focused on specific candidate genes/ different regions of the epigenome, which provides limited knowledge on the global nature of DNA methylation. Work on global DNA methylation has been limited in relation to

measures of anxiety and depression and studies that have, often report conflicting results.

In this study, we quantified global DNA methylation using the LINE-1 repetitive element, as part of the long interspersed nuclear element family (LINEs). The method has been used as a proxy for global DNA methylation, given that LINE-1 constitutes up to 17% of the human genome. Findings suggest that psychological distress, as reported by parents or children themselves, predicted variance in global LINE-1 DNA methylation in blood or buccal samples. The study has several strengths including the largest sample size of this study kind to date, as well as the use of dimensional measures of psychological distress. Findings suggest that psychological distress, as reported by young adolescents and their parents, did not significantly predict variance in global DNA methylation, as profiled from blood or buccal samples. Given the non-significant findings, it is worth replicating this work in other datasets including extending to clinical samples.

Table 7.1 – Summary of key findings from empirical research chapters

Chapter	Dataset (Country)	Sample Characteristics	Study design	Measures used	Key findings
3	TWINS (Netherlands)	N = 250 Mean age (SD) = 22.51 (3.59)	Multivariate ACE latent factor twin model	Anxiety, IBI, HRV, BRS	• Negative correlations between anxiety and cardiovascular measures. Significant between anxiety-BRS • Significant heritability for IBI • No significant genetic and environmental correlations
4	COTASS (Sri Lanka)	N = 3948 Mean age (SD)	Bivariate ACE sex-limitation	Anxiety, HRQL	• Negative correlations between anxiety & HRQL, mostly driven by E in men

		= 42.92 (14.55)	twin models		• & women and C in women • Significant C influences in women, limited evidence for A • Significant C and E correlations
5	COTASS (Sri Lanka)	N = 2922 Mean age (SD) = 42.84 (14.56)	Bivariate ACE latent factor twin model Direction-of-causation (DoC) models	Psychological distress, Physical health	• Negative correlation between two latent factors • Significant A for psychological distress • Support for DoC model running from psychological distress to physical ill health
6	TEDS & SRS (United Kingdom)	N = 155 Mean age (SD) = 11.94 (1.32)	Mixed multiple regression Pearson's correlations Within twin difference correlations	MFQ, SDQ, Combined psychological distress (MFQ+SDQ), LINE-1 global DNA methylation	• Psychological distress did not predict variance in global LINE-1 DNA methylation in blood or buccal samples • No significant correlations between psychological distress and LINE-1 DNA methylation • Within-twin difference correlations non-significant

TWINS = Twin Interdisciplinary Neuroticism study. COTASS = Colombo Twin and Singleton study. TEDS = Twin's Early development Study. SRS = Social relationships Study. IBI = Inter-beat interval; HRV = Heart rate variability; BRS = Baroreflex sensitivity. A = Additive genetic effects; C = Common/Shared environmental effects; E = Unique environmental effects. ACE = Twin design modelling A, C and E effects. DoC = Direction of Causation. MFQ = Mood's & feeling's Questionnaire. SDQ = Strength's & Difficulties Questionnaire. LINE-1 = Long Interspersed Nuclear Element 1.

7.3. General limitations

I have discussed limitations associated with each research study in the corresponding

chapters. In this section, I highlight some of the key limitations to these research studies in

sum.

Self-report measures

With the exception of cardiovascular autonomic measures in chapter 3, all measures in this thesis were obtained using self-reports. This method has advantages, such as it being quick and relatively easy to administer across large samples that are required for genetically informed studies. Questionnaire data, however, relies solely on the participant and can introduce factors such as recall bias and may not be an objective measure of mental and physical wellbeing.

In chapter 3, we included a rater-bias component which accounts for the additional covariance between a twin's self-report and what is reported by the co-twin, separating rater bias and unreliability from the latent anxiety factor. This component, however, could not be modelled for each twin modelling analyses (chapters 4 and 5) based on methodological constraints. For these chapters, participants came from a Sri Lankan dataset, which could potentially introduce a different set of biases. For example, research suggests that in developing countries like Sri Lanka, mental health can carry additional cultural stigma and individuals may be more inclined to mask mental health difficulties as physical health complaints (Mascayano et al., 2015; Samarasekara et al., 2012). Hence, sociocultural barriers may have potentially hindered the reporting of mental health.

The objectivity and reliability of self-reports could also be questioned in chapter 6, whereby children/ young adolescents were expected to understand and report on their internalising problems. Nevertheless, research suggests that children as young as seven can be reliable sources to gauge psychological distress, especially given that these experiences are complex, subjective and often not visible to others (Norwood, 2007). We also include parental reports

on their children's internalising symptoms in this chapter, adding a multiple-informant angle. This, however, can give rise to additional challenges such as deciding which informant's perspective is best to use and whether total/composite scores would be a better option. A related limitation to self-report is the role of measurement error. In the twin studies, this effect is captured in the unique environmental component. This means that this component could potentially be inflated and must be interpreted in the context of potential error in measuring and reporting. Shared measurement error is also an important consideration, such that an individual with high psychological distress may also report other types of distress mentally and physically, potentially inflating correlations between variables.

Sample size

Although the sample sizes in this thesis are larger than possible comparison published studies, results suggest that this type of research can benefit from larger samples to refine findings. Many parameter estimates have large confidence intervals, especially the case for aetiological correlations between variables in the twin analyses. Recent GWAS studies with substantial sample sizes (>10,000) have detected genetic correlations between anxiety/depression with several phenotypes (Purves et al., 2020). Though a separate study design to the twin and regression analyses presented here, we may have albeit been underpowered to detect such effects in the empirical chapters.

A variety of guidelines and power tables exist to determine and improve statistical power in twin model fitting approaches, such as a minimum of 600 twin pairs to reject inappropriate alternative models and the addition of non-twin siblings to family designs (Martin et al.,

1978; Posthuma & Boomsma, 2000). There are additional considerations for statistical power in twin model fitting. First, as twin model fitting primarily uses likelihood ratio tests, power is normally discussed under the χ^2 distribution (as opposed to a normal distribution). Second, standard power analyses are more tailored towards linear models and may not capture the additional complexities that a twin context brings.

Recent explorations have allowed power simulations for univariate and bivariate twin models in OpenMx (Verhulst, 2017). The method indicated several considerations. For example, results suggest that the power to detect genetic correlations increase as the magnitude of additive genetic effects (A) and the magnitude of the genetic correlation (rG) also increases (**Figure 7.1**). In chapter 4 we did not yield genetic correlations between anxiety and health related quality of life, possibly due to the low influence of A on the traits and the non-significant genetic correlations obtained. Similarly, the method suggests that; the power to detect A influences depends on the level of common environmental influences (C) ; the ratio of MZ and DZ twins affects the power to detect A and C ; continuous variables are an advantage over binary and finally, that large sample sizes are required to detect genetic sex differences. The exploratory method outlined above, however, is currently limited to univariate and bivariate designs and yet to be extended to multivariate and increasingly complex designs, such as the direction of causation model employed in chapter 5.

Figure 7.1 Power to detect genetic correlations as the magnitude of additive genetic influences varies.
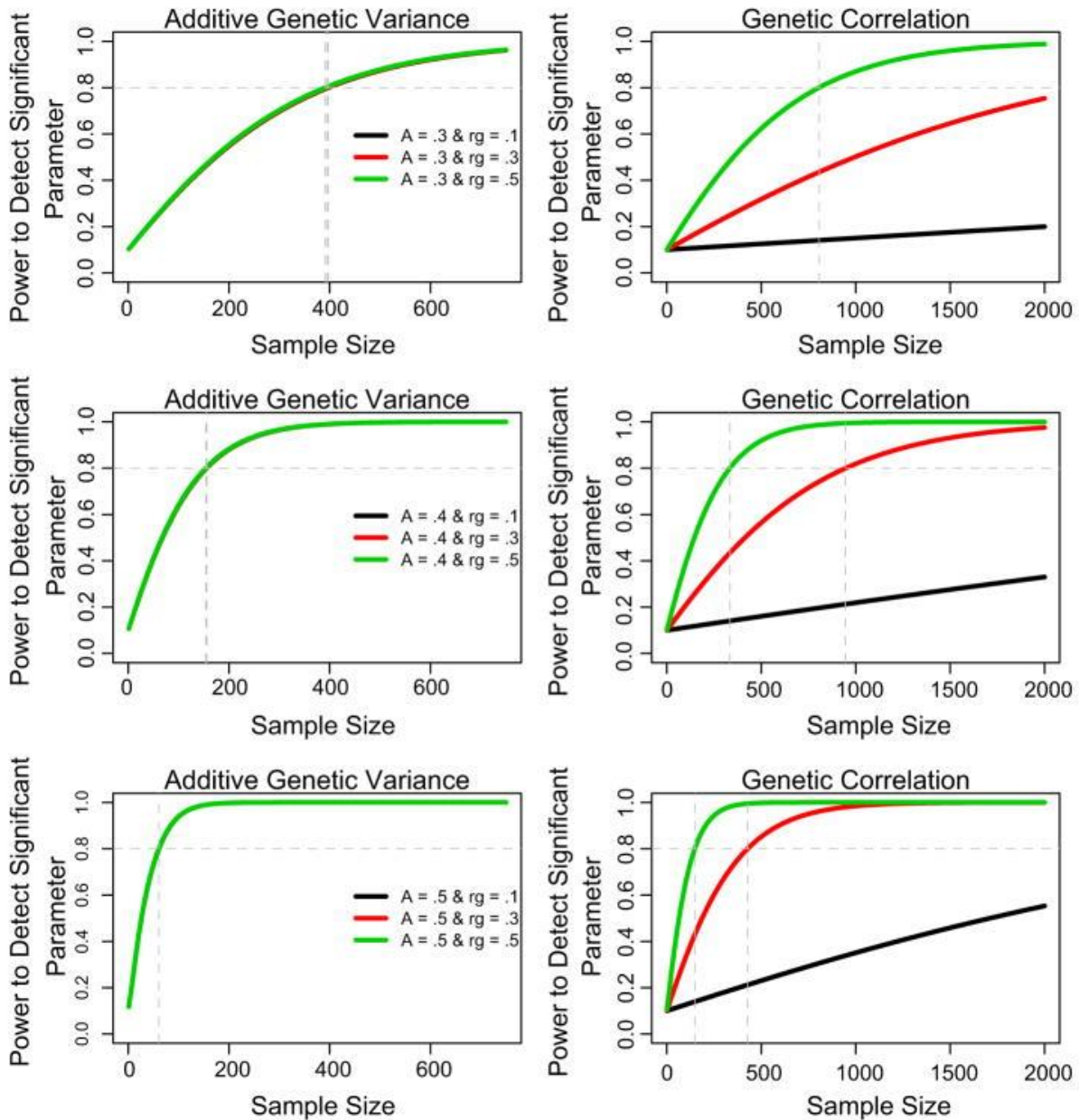

Figure taken from Verhulst (2017). A = Additive genetic influence; rg = Genetic correlation.

Non-clinical samples

Another potential limitation is that our samples are made up of individuals from the general

population, meaning that the results obtained here may not necessarily extrapolate to clinical

samples. To better understand the psychological distress spectrum, it is worth replicating this work in clinical settings with traditional diagnostic interviews. Although a potential limitation, the use of non-clinical samples may also pose an advantage in terms of capturing the dimensional nature of psychological distress as occurring in the population. In addition, as mentioned throughout this thesis, there is strong evidence for psychopathology being a quantitative continuum rather than strict categories/binary disorders (Krueger et al., 2018). Furthermore, this thesis also uses several ways to measure physical health which are not necessarily medical diagnoses. For instance, in chapter 3 we use three indices of cardiovascular functioning which are not sufficient to indicate whether individuals have/ may develop cardiovascular health problems. In addition, in chapter 5, we define physical health using four self-report items (general health, physical functioning, energy/fatigue and pain), which may not reflect the full spectrum of physical health issues. It is therefore worth noting that physical health in this thesis may be more suited as biomarkers/ symptoms of potential underlying physical health problems.

Cross-sectional data

The empirical studies in this thesis all feature a cross-sectional design, meaning that inference of causality is limited. The chapters presented here are therefore restricted to the concurrent aetiology and relationships between variables. It is quite possible that the aetiology and associations between measures of psychological distress and physical health show age-related, developmental changes. This is especially relevant to chapter 3, where cardiovascular autonomic measures can show drastic changes in older adulthood or in elderly populations where other physical health problems may also become apparent (Katayama et al., 2015;

Pinheiro et al., 2015). Cross-sectional twin data, however, can sometimes be informative in understanding direction of causation, as illustrated in chapter 5. Yet, there are possible limitations to this such as not knowing the true direction of causation (see chapter 5). It is therefore necessary to use a combination of statistical methods to infer causality and capture age-related genetic, epigenetic, and environmental influences (see future directions).

Twin modelling limitations

There are also possible limitations that are inherent to the twin design, discussed extensively in chapter 2 and throughout empirical chapters 3,4 and 5. For example, the generalisability of twin samples to the general population have been questioned with twins showing differences in prenatal environment and obstetric complications (Papiernik et al., 2010). In addition, it is possible that MZ twins are treated more similarly than DZ twins (violating the equal environments assumption and inflating genetic effects) as well as the role of non-random sexual selection (violating the 'no assortative mating' assumption, inflating shared environmental effects). As mentioned before, violation of these assumptions is unlikely to have large effects on parameter estimates (Andrew et al., 2001; Beijsterveldt et al., 2016; Derks et al., 2006). It would albeit be worthwhile, extending this work to other genetically sensitive methods (e.g., Genome-wide complex trait analysis, GCTA) that do not rely on assumptions of the twin method and consequent limitations.

DNA methylation method

We primarily discuss and use DNA methylation as an epigenetic marker in this thesis. There are various other types of epigenomic changes including histone and chromatin

modifications, which could have potential associations with psychological distress. In addition, we use the LINE-1 repetitive element to gauge global DNA methylation which has its limitations (chapter 6). Nevertheless, DNA methylation is one of the best studied epigenetic modification and global DNA methylation as profiled through repetitive elements can be a valuable biomarker for psychopathology (M. Li et al., 2019; Misiak et al., 2019; Moore et al., 2013). Further work may also benefit from using a combination of different methods to gain a more comprehensive understanding of the epigenome in relation to anxiety and depression.

7.4. Future directions

Molecular genetic research

The interface of mental and physical health is an exciting research field with emerging findings. One immediate future direction for this type of research is extending to molecular genetic research. The Psychiatric Genomics Consortium have established working groups for both anxiety and depression and GWAS results are beginning to identify top hits for measures of psychological distress, both in symptom and clinical forms (Howard et al., 2019; Purves et al., 2020). Possible future work may also include a deeper exploration of the physical aspects and consequences of these disorders as well as integrating other 'omics' data such as epigenetics.

Extensions of the Twin Design

As mentioned above, a major limitation involves the cross-sectional nature of the studies, limiting causal inference. Although in some cases cross-sectional twin data can help with

determining causal direction, longitudinal studies are an essential tool that can be used to track mental and physical wellbeing overtime. The *Cholesky decomposition* for the twin design can be a useful method to begin disentangling these longitudinal effects, and to explore age-related genetic and environmental effects. This will also be useful in deciphering the stability of these effects overtime, and whether there are specific developmental windows that can be used for intervention purposes. For instance, if non-shared environmental effects start to become more influential on the mental-physical health relationship, these factors (e.g., work-related stress, family factors) could potentially be identified and conditions be improved.

Longitudinal twin studies can therefore be an imperative tool to model variables that have a temporal order. For instance, if trait Y is measured after trait X, it is feasible to model so that X predicts Y and not vice versa. This temporal order of measurement, however, is not justifiable in all cases (McAdams et al., 2021). For example, if psychological distress is measured at time 2 and physical health at time 1, this may not be sufficient rationale to model physical health predicting psychological distress. Ideally, both psychological distress and physical measures should be measured at the same time so that we can investigate whether poor physical health prospectively predicts psychological distress after accounting for the correlation between the two measures at time 1 and stability in physical health between time 1 and time 2.

Autoregressive cross-lagged models can also be a future research direction and an alternative to the abovementioned limitation of longitudinal twin studies. Briefly, the model investigates

the longitudinal effect of one trait on another above and beyond the stability of each trait (e.g., from time point 1-2) and the concurrent correlation between traits (at time point 1 and 2). This can be extended to biometric models whereby it can be estimated whether trait X (measured at time 1) predicts trait Y (time 2) after accounting for genetic and environmental influences common to these two variables and influences on Y (time 1). It is also possible to disentangle the cross-lagged paths into genetic and environmental influences. The model has been previously used to explore links between reading motivation and achievement (Malanchini et al., 2017), between anxiety and depression (Tanguay-Garneau et al., 2020; Zavos et al., 2012) and between internalising behaviour and parental involvement (Moberg et al., 2011). This model could be extended to the context of mental-physical health. For instance, exploring longitudinally whether the direction of causation runs from psychological distress to poor physical health, as mentioned in chapter 5, whilst accounting for their aetiology, correlations, and stability overtime.

Additionally, an exciting direction of research involves extending the twin design to incorporate molecular genetic information. For instance, recent work combines mendelian randomisation (MR) with the direction of causation (DOC) model presented in chapter 5. MR has become an imperative tool to establish causality in observational/non-experimental research. The method is primarily based on the use of genetic variants (e.g., SNPs) as instrumental variables to test causal associations between exposures/risk factors (e.g., stressful life events) on outcomes (e.g., physical, or mental health). The method, however, has various assumptions to be met, including a strong relationship between instrument and exposure (whereas SNPs have relatively weak effect sizes) and even if these variants are

combined into a polygenic score, this renders the assumption of no pleiotropy of the instrument (whereas a polygenic score is likely to also influence the outcome). The *MR direction of causation model (MR-DOC)* (Minică et al., 2018) incorporates the polygenic score method within the DOC twin design, allowing unbiased testing of causal hypotheses and can directly test the pleiotropic nature of variables. The model could strengthen inferences on causality by pinpointing specific genetic variants that could be causal in the mental-physical health relationship.

Furthermore, a natural next step from this research is the use of extended family designs, such as the *children-of-twins (CoT) model* (McAdams et al., 2018). The method is discussed elsewhere in more detail (D'Onofrio et al., 2003; Silberg & Eaves, 2004). Briefly, the method compares MZ avuncular correlations (correlations between uncle/aunt and niece/nephew) with DZ avuncular correlations, which can estimate the role of genetic factors in explaining intergenerational associations. The CoT design also allows for estimating the extent to which parent–child associations remain after accounting for genetic transmission. This extended twin method has been used to gauge associations between children and their parents in the context of anxiety and depression (Ahmadzadeh et al., 2021; Eley et al., 2015). This design could also be extended to incorporate measures of physical health, adding a new layer of knowledge on how mental-physical health can manifest intergenerationally. The CoT design could also be useful in disentangling gene-environment correlations, introduced in chapter 1, and strengthen causal inferences. This highlights the long-lasting value of the twin design in the modern era of quantitative genetics (van Dongen et al., 2012), and further ways to explore the mental-physical health interface.

Epigenetics

As mentioned previously, longitudinal designs are an important tool for research. It is also known that the epigenome is dynamic, meaning that new epigenetic changes may arise overtime (Kane & Sinclair, 2019; Y. Li & Tollefsbol, 2016). For instance, research indicates that environmental triggers such as bullying, and victimisation have been associated with DNA methylation changes (Kandaswamy et al., 2020; Mulder et al., 2020). As such, future work on the epigenetic nature of anxiety and depression can also benefit from these longitudinal designs.

There are several other possible future avenues for epigenetic discovery. In terms of global DNA methylation, an immediate future direction would be the investigation of other repetitive elements/ retrotransposons in relation to anxiety and depression. For example, with over 1.4 million copies in the genome (Reszka et al., 2021), *Alu elements* are short interspersed nuclear elements that may be another potential proxy to gauge global DNA methylation. Research has highlighted the role of Alu elements in relation to phenotypes such as schizophrenia and post-traumatic stress disorder (Misiak et al., 2015; Rusiecki et al., 2012) and extending this to anxiety and depression may be a fruitful approach.

In addition to global DNA methylation, future work can benefit from *epigenome-wide approaches* to understand psychological distress. With new developments in epigenome-wide association studies we may be able to discover new differentially methylated regions in those with an anxiety or depressive disorder. In addition, even the largest epigenomic studies are currently limited to arrays that cover approximately 500-800,000 CpG sites, which is a

fraction of over 20 million CpG sites across the genome. Future technological advances can mean that studies can become increasingly comprehensive in terms of coverage.

Integrated Healthcare

Ultimately, research on the associations between mental and physical health has clinical implications. Traditionally, the public views and receives treatment for their psychological health separately to their physical health problems. This thesis provides additional evidence for their co-occurrence and supports the notion of holistic healthcare. The World Health Organisation proposed an objective in their comprehensive mental health action plan (2015-2020) for all countries: 'to provide comprehensive, integrated, and responsive mental health and social care services in community-based settings' (Saxena et al., 2015). The Lancet Psychiatry commission has also provided recommendations for an integrated healthcare approach (**Figure 7.2**) (Firth et al., 2019). Yet, progress has been slow. Although not new, implementing an integrated health approach often has practical and financial drawbacks (Kathol et al., 2010).

Integrated healthcare efforts have been made in China through the '686 project', which aimed to a) establish an integrated identification and treatment system for those with mental illnesses; b) increase treatment rates for serious mental illnesses; c) increase community awareness about the characteristics and treatment options; d) increase the rates of successful recovery and rehabilitation; and e) alleviate the difficulties of patients and their family members (Ma, 2012). Other approaches have included the Diabetes Prevention program (DPP) in the USA. This included frequent face-to-face contact with participants, structured

educational components including behavioural self-management strategies, supervised

physical activity sessions, tailoring of materials and strategies to address ethnic diversity, and

an extensive network of training, feedback, and clinical support (Diabetes Prevention

Program (DPP) Research Group, 2002). Yet, several barriers remain, including the shortage

of mental health workers, lack of routine screenings, the need for additional training and

reaching to individuals in rural areas (Liang et al., 2018). There is also the need for effective

and well-managed health record and referral systems and high level of communication

between departments and healthcare facilities (Funk, 2008). This leads to a further challenge:

integrating mental and physical health in developing and low-middle income countries

(LMICs).

Figure 7.2 Recommendations from the Lancet Psychiatry commission on how to integrate mental-physical health
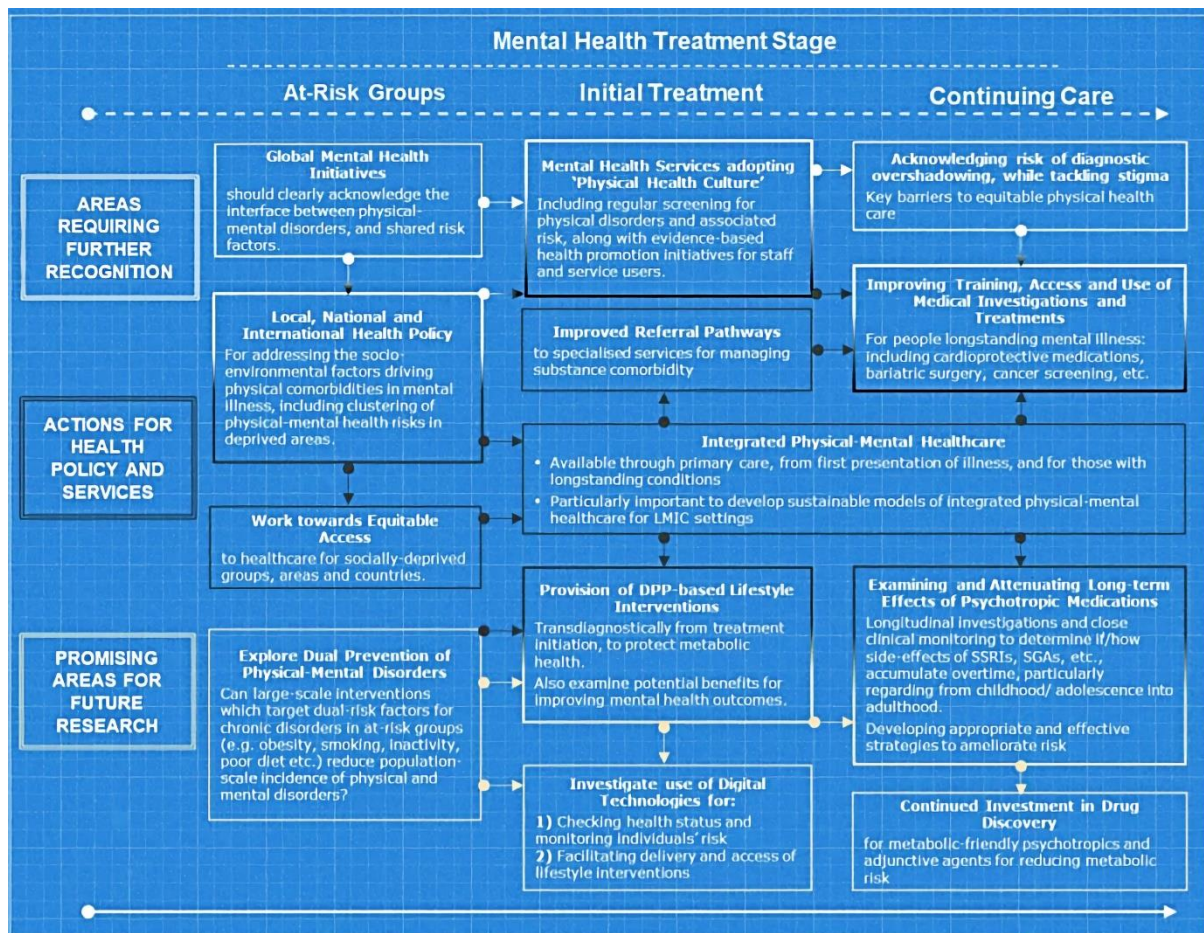

Figure taken from Firth et al (2019). Note: Box placement on X-axis represents 'start point'; i.e., applicable from that point in mental health stage, and onwards. Box placement (and line colouring) on Y-axis represents overlap with 'areas' for greater recognition, clinical actions, and future research. DPP= Diabetes Prevention Program, a lifestyle intervention founded in the USA. SSRIs = Selective Serotonin Reuptake Inhibitors; SGAs= Second Generation Antipsychotics. LMIC = Low-Middle Income Country.

Research suggests additional barriers to an integrated health approach present in LMICs. In many such countries, mental healthcare is often poorly governed and implemented (Semrau et al., 2019). Other barriers include barriers in coordination (e.g., across government levels and higher levels of leadership), human resources and skill (e.g., insufficient capacity to translate policy to action, unpreparedness), inappropriate/ill-fitting policies (e.g., policies

developed externally/ in developed countries that do not account for local community and spiritual/religious beliefs), leadership and accountability (e.g., authoritarian leadership, poor commitment to mental health) and financial factors (lack of funding and finance allocated to either mental or physical health rather than an integrative way) (Thornicroft et al., 2019). Of the few studies conducted on integrated healthcare in LMICs, findings are in favour and suggest multiple benefits including improved patient detection, coordinated care, decreased stigma, and improved mental health treatment outcomes (Thornicroft et al., 2019). It is recommended that further research and funding into mental-physical health can provide long-term benefits and allow culturally appropriate implementation in LMICs (Lempp et al., 2018).

Genetic research in non-western populations

Following from this, a broad future avenue is the inclusion of more diversity within behaviour genetics and science in general. The lack of ethnic diversity and the focus on mostly European populations has been a weakness of genetic research thus far (Manolio, 2019; Popejoy & Fullerton, 2016). The under-representation of ethnically diverse populations hinders the ability to fully understand the genetic architecture of human health and disease and could exacerbate existing health inequalities. Ultimately, the lack of ethnic diversity in human genetic research means that translation of this research into clinical settings or public health policies may be 'dangerously incomplete, or worse, mistaken' (Sirugo et al., 2019). For example, genotyped datasets often recruit participants through case-control studies, volunteer biobanks and direct-to-consumer companies, which can mean individuals can differ on several sociodemographic and health factors than the population average (Fry et al., 2017).

In contrast, twin studies are viewed as more representative of populations and less likely to be affected from these selection biases (Mostafavi et al., 2020). In this thesis, I have used the Sri Lankan sample mentioned in chapters 4 and 5 which is one of its kind in terms of a twin and singleton registry in a South Asian sample. However, with most twin studies aggregating in western populations (Polderman et al., 2015), there is an ongoing need for more twin registries worldwide.

Representation of non-western populations, especially nations that are less economically developed, can provide new insights into the genetic, epigenetic, and environmental factors underlying complex traits. For instance, in chapter 4, the influence of shared environment is found to be large and significant for anxiety and several health related QoL measures, whereas this component is often negligible in twin studies from western populations. In addition, this can provide further information on how the relationship between mental-physical health manifests in different populations. This is especially relevant for groups that have been historically marginalised and/or discriminated against based on unfounded beliefs on biological differences between groups (Fitzgerald, 2014; Popejoy et al., 2018). Going forward, representativeness of genetic research can be improved through building trust (Hindorff et al., 2018) and recruitment of large and ancestrally diverse samples. There is hope for the future, with twin registries being established across the world (Hur et al., 2019) and cohorts such as the East London Genes & Health project with genotype data for over 38,000 participants of South Asian ancestry (Finer et al., 2020). This type of work will not only improve the quality of genetic research but also allow subsequent inferences drawn to be applicable to people of all backgrounds. Most of the world is made up of non-western nations

and it is essential that scientific discovery reflects this.

To conclude, this thesis uses genetically and epigenetically informative designs to infer the aetiology of psychological distress and its associations with markers of physical ill health and health-related quality of life. Ultimately, this research, coupled with collaborative efforts across the world, can inform on clinical practice. It is time to view health holistically and identify and treat mental and physical health problems together.

7.5. References

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