Investigating Shared Genetic and Environmental Aetiology between Psychiatric Disorders and Rheumatoid Arthritis

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Abstract

Complex diseases are defined by having a multifactorial aetiology, consisting of multiple genetic and environmental risk factors. Complex diseases are often associated with unusual patterns of comorbidity. They are also typified by suboptimal nosology, being classified according to historical diagnostic boundaries that may not be strongly justified given emerging evidence on pathophysiology. Epidemiological studies have shown an unusual pattern of comorbidity between the psychiatric and autoimmune disorders – two broad categories of complex disease, however the aetiology underlying this overlap is yet to be established.

We present three investigations into the overlap between the psychiatric and autoimmune disorders. First, we review the epidemiological literature of the phenotypic relationship between schizophrenia and rheumatoid arthritis and perform a meta-analysis of studies meeting inclusion criteria. Next we investigate evidence for an enrichment of schizophrenia genetic risk amongst controls for rheumatoid arthritis using a number of existing statistical genetic techniques. We find no evidence that common genetic variation influences the low prevalence of rheumatoid arthritis in schizophrenia cases.

In a longitudinal population cohort we model depression genetic risk and its influence on the onset of depression, autoimmune disorders, and the comorbidity between the two. We find evidence that autoimmune disorder onset increases the risk of subsequent depression onset, independent of depression genetic risk.

In a cohort of rheumatoid arthritis patients, we investigate the role of depression genetic risk and rheumatoid arthritis severity on disease progression. We find that low mood is a significant predictor of worse treatment outcomes, including inflammatory components of rheumatoid arthritis disease severity.

To interrogate the genetic aetiology underlying comorbidity, we extend the polygenic risk score (PRS) approach in two ways. First, we develop software, PRSice, to perform PRS analyses. Secondly, we develop a novel PRS method to calculate PRS in cross-disorder scenarios.

Statement of Authorship

All work in this thesis was carried out and written by Jack Euesden, with the exception of the following;

Chapter 2:

- The RADIANT genotypes, which are used in the validation of our method, were collected, genotyped and cleaned by others prior to analysis

Chapter 3:

- The WTCCC and RADIANT genotypes were collected, genotyped and cleaned by others prior to analysis

Chapter 4:

- The NCDS genotype and phenotype data used in this analysis was collected, genotyped and cleaned by others prior to analysis

Chapter 5:

- The CARDERA cohort was collected, genotyped and cleaned by others prior to analysis. Ian Scott provided invaluable expertise on the clinical presentation of rheumatoid arthritis throughout the writing of this chapter

Chapter 6:

- In addition to using the RADIANT genotypes discussed above, the UK Biobank data was collected, genotyped and cleaned by others prior to analysis
- The simulation software used in this chapter to validate the performance of our method was developed by Yunfeng Ruan, under the supervision of Paul O'Reilly and Jack Euesden

In addition to the above, the papers in this thesis – submitted or published - were circulated around co-authors and underwent peer review, leading to editing of manuscripts and suggestions on additional analyses

Eusden, a laurel'd bard, by fortune rais'd,
By few been read, by fewer still been prais'd

Cooke, The Battle of the Poets, 1725

Know, Eusden thirsts no more for sack or praise;
He sleeps among the dull of ancient days;

Pope, The Dunciad, 1728

List of abbreviations

Phenotypes	
AD	Autoimmune Disorder
BMI	Body Mass Index
CD	Crohn's Disease
HDL	High-Density Lipids
LDL	Low-Density Lipids
MDD	Major Depressive Disorder
RA	Rheumatoid Arthritis
SCZ	Schizophrenia
T1D	Type 1 Diabetes
UC	Ulcerative Colitis
Genetics	
GRM	Genetic Relatedness Matrix
HLA	Human Leukocyte Antigen
LD	Linkage Disequilibrium
MAF	Minor Allele Frequency
MHC	Major Histocompatability Complex
SNP	Single Nucleotide Polymorphism
Methods and Statistics	
ANOVA	Analysis of Variance
CI	Confidence Interval
GREML	Genomic Relatedness Matrix Restricted Maximum Likelihood
GWAS	Genome-Wide Association Study
HR	Hazard Ratio
IRR	Incidence Rate Ratio
LDSC	LD Score Regression
MDS	Multi-Dimensional Scaling
OR	Odds Ratio
PCA	Principal Component Analysis
PRS	Polygenic Risk Score
QC	Quality Control
SD	Standard Deviation
VIF	Variance Inflation Factor
Cohorts and Consortia	
CARDERA	Combination Anti-Rheumatic Drugs in Early RA trial
GIANT Consortium	Genetic Investigation of Anthropometric Traits Consortium
GLC	Global Lipids Consortium
ISC	International Schizophrenia Consortium
NCDS	National Child Development Study
NFBC	Northern Finland Birth Cohort
PGC	Psychiatric Genomics Consortium
TAG	Tobacco and Genetics Consortium
TEDS	Twins Early Development Study
UKBB	UK Biobank
WTCCC	Wellcome Trust Case Control Consortium

Chapter 1: Introduction

Primary hypotheses

There is considerable evidence for an autoimmune or inflammatory component to risk of psychiatric disorders, and this may be directly related to the patterns of comorbidity observed between these families of disorders. Within the most intensively studied psychiatric disorders - such as schizophrenia - evidence for an autoimmune component to disease is growing (Khandaker, Pearson, Zammit, Lewis, & Jones, 2014; Wium-Andersen, Orsted, & Nordestgaard, 2014a) and may guide the development of new pharmacological interventions in the future.

The aim of this thesis is to investigate causes of the overlap between the psychiatric disorders – with a focus on depression and schizophrenia – and the autoimmune disorders – with a focus on rheumatoid arthritis – at an epidemiological and genetic level. Our primary hypotheses concern the phenotypic relationships observed between the autoimmune and psychiatric disorders. Firstly, that these are genuine and not confounded by a systemic bias such as a harvesting effect - whereby individuals with schizophrenia and higher vulnerability towards rheumatoid arthritis, perhaps due to a risk factor such as smoking or urbanicity, may have a higher mortality rate the therefore be less likely to live to age at onset for rheumatoid arthritis - a reporting bias or a treatment effect. Secondly, that these relationships are due to some common aetiological factor that has pleiotropic effects - that is to say, has downstream effects influencing both psychiatric and autoimmune disorders, perhaps in different directions. This may act at a genetic level, where a common genetic risk profile is responsible for comorbidity

between two phenotypes. Alternately, it may act at a physiological level, where a particular perturbation of a biological pathway results in multiple downstream phenotypes. Therefore our primary hypotheses are firstly that the overlap between psychiatric and autoimmune disease isn't simply a result of confounding. Secondly, if an overlap is present, it would necessarily be due to shared risk factors; if genetic, these would be pleiotropic. Thirdly, we hypothesise that evidence for shared environmental risk factors can be supported via the rejection of the hypothesis that there is a genetic component to epidemiological relationships. Fourthly, we test the hypothesis that the identification of shared genetic risk can be performed currently by a number of available tools. Finally, we test whether these may be improved via novel methods and approaches.

We will investigate these hypotheses using individual-level genotype data, genome-wide summary data for association with a phenotype, and phenotypic data detailing the time-course of a phenotype and its relationship with the environment. Finally, we will extend current methods in a number of ways to develop more accurate predictors of genetic risk, which may in turn aid in our understanding of the genetic architecture of comorbid complex phenotypes.

Phenotypes Under Investigation

Autoimmune Disorders

The autoimmune disorders are a class of physical disorders typified by a failure of 'self tolerance'. The Mammalian 'active' immune system kills cells it encounters by default; tolerance of 'self' cells, recognised by proteins in their cell membranes, prevents destruction of 'self' tissue. A breakdown in this learned self tolerance defines the

pathophysiology of the autoimmune disorders. Here we will focus on rheumatoid arthritis as an example of the autoimmune disorders. We do this firstly due to its relatively high prevalence – 552 per 100,000 in a Sardinian population (Sardu et al., 2012), the second highest prevalence autoimmune disorder reported by the authors after autoimmune thyroiditis, and 860 per 100,000 in a meta-analysis of studies in the USA (Jacobson, Gange, Rose, & Graham, 1997) the second most common reported after Graves disease. Secondly, rheumatoid arthritis is associated with a comparatively short time lag between onset and diagnosis – median time 36 weeks (Chan, Felson, Yood, & Walker, 1994) – which will effectively minimise undiagnosed cases in the population and thus improve the power of cohort studies and those relying on self-report. Indeed, in a cohort of 2458 pregnant women, deep phenotyping revealed a prevalence of undiagnosed rheumatoid arthritis of 0.24% (Spinillo et al., 2012). This contrasts to, for example, celiac disease, where authors have found that 95.6% of cases in a Netherlands-based study were undiagnosed (Schweizer, von Blomberg, Bueno-de Mesquita, & Mearin, 2004). Finally, although rheumatoid arthritis is associated with a relatively late age at onset – mean of 58.0 (Cooper & Stroehla, 2003; Doran, Pond, Crowson, O'Fallon, & Gabriel, 2002) – however the authors report a standard deviation of 16 years, producing wide estimated confidence intervals (95% CI = 26.6 – 89.4). This has the effect of leading to a relatively high incidence rate of rheumatoid arthritis per 100,000 person years – 17 before the age of 16 and 23.7 thereafter – the highest incidence rate reported in a review of autoimmune disorder incidence rates (Cooper & Stroehla, 2003). These epidemiological properties – in addition to its well-studied genetic component, discussed below - make rheumatoid arthritis especially amenable to study via population cohort methods compared to other autoimmune disorders.

Psychiatric Disorders

The psychiatric disorders are defined here as medical disorders – measurable and impairing deviations from normality – that affect an individual's behaviour without a measurable physical aberration. The psychiatric disorders represent a substantial burden to societies worldwide, however their genetic components have only been intensively studied relatively recently. A recent report shows that MDD is the leading cause of disability worldwide (World Health Organisation, 2012).

Epidemiological Relationships between Psychiatric and Autoimmune Disorders

Schizophrenia and the autoimmune disorders

There are a number of studies investigating the phenotypic overlap between schizophrenia and the autoimmune disorders. Benros et al present a detailed (table 1), investigating many of these epidemiological relationships in a Danish population cohort (Benros, Pedersen, et al., 2014). Despite the aggregate tendency towards comorbidity between schizophrenia (SCZ) and any autoimmune disorder, the authors find varying effects with different disorders. They find no evidence for a relationship between SCZ and ulcerative colitis, and an apparently protective relationship between SCZ and rheumatoid arthritis (RA).

In a meta-analysis of previous literature, we demonstrate evidence that this protective effect of schizophrenia on RA is consistent across prior studies (Euesden, Breen, Farmer, McGuffin, & Lewis, 2015), (chapter 3). A number of explanations have been proposed for this relationship, most notably the well-established anti-inflammatory activity of antipsychotic medication.

MDD and the autoimmune disorders

Odegaard's dictum (Odegaard, 1952) - that all schizophrenia sufferers eventually pass through a hospital - makes SCZ much more amenable to large population-based studies of its epidemiology, compared to MDD. Nevertheless, a few studies of MDD and its overlap with autoimmune disease have been conducted. In order to compare the overlap between schizophrenia and the autoimmune disorders with evidence for an overlap between MDD and the autoimmune disorders, we discuss these with reference to the disorders investigated in conjunction with SCZ above. Similar findings to Benros et al (2014) have been observed in multiple sclerosis (Patten, Beck, Williams, Barbui, & Metz, 2003), type 1 diabetes (Anderson, Freedland, Clouse, & Lustman, 2001), ulcerative colitis - when preceding MDD onset by under 1 year (Kurina, Goldacre, Yeates, & Gill, 2001), Crohn's disease (also when preceding MDD by under a year, Kurina et al 2001) and seropositive rheumatoid arthritis (Dickens, McGowan, Clark-Carter, & Creed, 2002), who calculated a phenotypic correlation rather than an odds ratio, by meta-analysis (summarised in table 2).

It is not immediately clear why there is limited research on the epidemiological patterns of disorders comorbid with MDD, when large record linkage studies in schizophrenia are frequently published. In part this may be due to the comparatively poor detection of MDD in primary care (Farmer & Griffiths, 1992; Lane, Shellenberger, Gresen, & Moore, 2000). This in turn necessitates deep phenotyping of cases – to maximise power – and controls – to prevent misclassification.

Experimental evidence for Shared Pathways between Psychiatric and Autoimmune Diseases

A natural extension of the above epidemiological results is an extension of the biological mechanisms responsible. It is often argued that the first evidence for an interaction between psychological and immune pathways came in 1982. Bovbjerg, Ader & Cohen investigated (1) the graft-host response - an immune response to donor tissue caused by non-self antigens stimulating cytotoxic T lymphocytes – and (2) classical conditioning - a form of implicit learning that is likely to be distributed across the synapses of the nervous system (Bovbjerg, Ader, & Cohen, 1982). By pairing a saccharine solution (a conditioned stimulus, CS) with injection of an immunosuppressant, Cyclophosphamide (CY, an unconditioned stimulus, US), rats developed a Conditioned Response (CR) to the CS, ultimately resulting in suppressed immune activity. Subsequent immune responses could be suppressed through the use of a CS, mimicking the immunosuppressant effect of CY. This important result - that immune responses can be modulated by psychological processes - provides a platform for understanding subsequent results regarding immune and autoimmune activity in MDD and SCZ.

Immunity in MDD

Herbert & Cohen demonstrated by meta-analysis that differential immune responses are a characteristic of MDD (Herbert & Cohen, 1993). The authors found reliable evidence for impaired immune activity (e.g. decreased lymphocyte proliferation in response to stimulation by a mitogen in vitro), and this has been reliably replicated in the two decades since. There is now an extensive body of literature investigating the pathophysiology of inflammation-related depression (A. H. Miller & Raison, 2016), with one proposed mechanism being the activation of the enzyme Indoleamine 2,3-dioxygenase (IDO) by

inflammatory cytokines, which catabolises tryptophan leading to a downstream depletion in serotonin (A. H. Miller & Raison, 2015) – indeed inflammation-related depression appears to dependant on the activation of IDO (O'Connor et al., 2009). In light of this, anti-inflammatory medication has been proposed as a treatment for inflammation-related depression

The immune system has a number of integrated pathways for neutralising external threats – here we will focus on the cytokine system and the innate immune system and discuss evidence for systemic differences between MDD patients and controls in these pathways. Firstly we will consider the cytokine system, a family of intercellular signalling molecules released by $CD4^+$ T-lymphocytes (helper cells) in response to stimulation by a protein identified as foreign - an *antigen* - in order to effect a downstream immune response. Secondly, we will consider elements of the innate immune system, the Pattern Recognition Receptors (PRR), that respond to stereotyped Pathogen Associated Molecular Patterns - that is to say proteins that form stereotyped parts of pathogen biochemistry and are therefore indicators of infection - and trigger the complement system.

Cytokines in MDD

Cytokines are signalling molecules released by an number of cells in the immune system that broadly fall into two classes - pro-inflammatory and anti-inflammatory. The pro-inflammatory cytokines have a number of physiological roles, and many have been reliably associated with MDD. Interleukin-6 (IL-6) activates the Hypothalamic-Pituitary Axis (HPA) - a component of the stress response - and has been found at elevated levels in the serum of MDD patients (Alesci et al., 2005). IL-1 β has been found at increased concentrations in MDD patients versus controls (Schlatter, Ortuno, & Cervera-Enguix,

2004). In tandem with this, Anisman et al showed that IL-1 β levels could actually predict HAM-D scores (a measure of MDD symptom severity) in MDD patients (Anisman, Ravindran, Griffiths, & Merali, 1999). Similarly, Tumour Necrosis Factor α (TNF- α) has been found at elevated levels in MDD patients' serum (Tuglu, Kara, Caliyurt, Vardar, & Abay, 2003).

The Innate Immune System in MDD

C-reactive protein (CRP) is a PRR that responds to phosphocholine, a marker of bacterial infection, forming part of the innate immune system. CRP has been found at elevated serum levels in MDD patients (Ford & Erlinger, 2004) and in male MDD patients (Danner, Kasl, Abramson, & Vaccarino, 2003). Mendelian randomisation is a method that uses genetic data to infer causality from phenotypic correlations. Wium-Andersen et al apply Mendelian randomisation to the association between MDD and CRP levels; the authors argue that this relationship is actually merely correlational, with some shared risk factor leading to both increased risk of depression and increased CRP levels (Wium-Andersen, Orsted, & Nordestgaard, 2014b). Despite this, there is evidence that the CRP system is influenced by many other depression-related factors, including obesity, (Daly, 2013), CBT response, (Keri, Szabo, & Kelemen, 2014) and physical exercise (Eyre, Papps, & Baune, 2013). Furthermore, Wium-Andersen et al's results should be viewed relatively sceptically – the authors present a negative result with no power calculations, suggesting their conclusions may be unsupported. Additionally, mendelian randomisation carries a number of limitations that must be adhered to strictly in order to ensure that results are interpretable (Davey Smith & Hemani, 2014). In summary, it is likely that studying the innate immune system will improve our understanding of the biological processes underlying the pathophysiology of MDD.

Immunity in Schizophrenia

Whilst serological studies of MDD have focussed on the cytokines - signalling molecules released by many types of immune cell - serological studies of SCZ have focussed on antibodies. Antibodies – or Immunoglobulins (Ig) - are large protein complexes, released by B-lymphocytes, which fall into a number of categories denoting the specificity with which they bind antigens. Antigen is an operationalised term that defines proteins that antibodies bind to, and antigens expressed on the membranes of pathogens are important in eliciting an adaptive immune response. This binding leads to a number of downstream events (Porter, 1959) including marking invading cells for destruction via the complement system, which triggers the destruction of marked cells. One of the first studies to demonstrate a link between antibodies and SCZ (McAllister et al., 1989) found that $CD5^+$ B-lymphocytes were elevated in the serum of SCZ patients at a level comparable with that observed in rheumatoid arthritis patients. $CD5^+$ B-lymphocytes secrete Immunoglobulin M (IgM), the largest of the basic antibody families. Steiner et al found an elevated concentration of B-lymphocytes (using a less specific $CD19^+$ assay), although the authors did not assay for $CD5^+$ cells (Steiner et al., 2010). They authors also investigated T-lymphocyte levels and the $CD4^+/CD8^+$ ratio - a marker of immune regulation - however these results did not survive correction for multiple testing and will not be discussed here. Possibly the most compelling summary of the antibody literature in SCZ can be found work by Ezeoke et al (Ezeoke, Mellor, Buckley, & Miller, 2013), who demonstrated by meta-analysis that self-reactive antibodies for the N-methyl-D-aspartate (NMDA) receptor are elevated in SCZ patients – summarised in table 3. This integrates with the B-lymphocyte literature above and the psychiatric nature of SCZ. The NMDA receptor is involved in the maintenance of learned behaviour within neural circuits (Bannerman, Good, Butcher, Ramsay, & Morris, 1995), and so it is possible to

tentatively infer a pathway from the cellular abnormalities observed in the serum of SCZ patients to the behavioural differences.

Genetic Determinants of Common Disease

Identifying the genetic contribution to common disease

Statistical genetic techniques can be used to characterise biology. Genome-wide association studies (GWAS) aim to identify relatively common genetic variants associated with a given phenotype (Lewis & Knight, 2012). The most recently published GWAS of rheumatoid arthritis (Okada et al., 2014), the largest to date, was performed on 29,880 RA cases, 73,758 controls and over 10 million loci studied. Such a large sample size provides high statistical power and increases the probability of identifying single nucleotide polymorphisms (SNPs) with only modest individual contributions to disease status; SNPs are polymorphisms at a single nucleotide, and are the genetic predictors used within GWAS. SNPs under investigation are usually restricted to those with a frequency in the population (Minor Allele Frequency, MAF) above some value, typically 1%. Studying diseases with high heritability also increases the power of GWAS. Heritability is defined here as the proportion of variance in a trait attributable to genetic factors – and SNP heritability, a special case of this, is variance attributable to common SNPs, i.e. the heritability that can be identified through GWAS. Cases and controls are genotyped on a chip for approximately half a million common SNPs, and each SNP is tested in a univariate regression model for its prediction on case status. Due to the large multiple testing burden inherent in this paradigm, a conservative statistical significance threshold of $\alpha = 5x10^{-8}$ is typically applied (Dudbridge & Gusnanto, 2008). Furthermore, under natural selection, we implicitly expect genetic variants with a relatively high frequency in the population to have such minor effects on disease risk that they are

relatively invisible to selection. Due to these concerns amongst others, sample sizes in excess of the tens of thousands are usually required for GWAS.

The genetics of autoimmune disorders

Many autoimmune disorders have high heritabilities – for example, as estimated from twin studies, rheumatoid arthritis has a heritability of 53% (95% CI = 40-65%) in a UK sample (MacGregor et al., 2000). Most – but not all – of this heritability can be explained by haplotype sharing at the HLA region (Deighton, Walker, Griffiths, & Roberts, 1989), which is estimated to have a heritability of 37%. This is not reported with confidence intervals, however this is consistent with studies of common genetic variation genome-wide excluding the HLA region. Stahl et al use GREML – discussed below – to estimate that rheumatoid arthritis has a SNP-heritability, i.e. heritability due to variants that can be identified by GWAS, of 32% (SE = 3.7%) (Stahl et al., 2012). The authors also find a SNP heritability for celiac disease of 33% (SE = 4.2%). Thus this high heritability of the autoimmune disorders has led to intensive investigation into their genetic determinants in order to develop more appropriate treatment for these chronic disorders. To date, four of the most widely studied autoimmune disorders are ulcerative colitis, Crohn's disease, type 1 diabetes and rheumatoid arthritis (UC, CD, T1D, RA). GWAS have been applied to these four disorders with considerable success, identifying between 41 and 101 loci reaching genome-wide significance, in type 1 diabetes and rheumatoid arthritis respectively (Barrett et al., 2009; Okada et al., 2014). Consequently, this has led to an increased understanding of the biological pathways involved in these disorders. All four of these disorders are chronic, that is to say incurable and on-going, and are managed through a number of immunosuppressant drugs alongside sometimes radical lifestyle modifications. The economic burden of such phenotypes makes them natural targets for

medical research; it is hoped that an increased understanding of the biological pathways perturbed in these disorders will guide drug discovery and prophylactic initiatives.

The genetics of rheumatoid arthritis

The large number of validated loci reaching genome-wide significance for rheumatoid arthritis – 101 - allowed Okada et al to run a series of pathway-based analyses to understand these GWAS results in a biological context. By using epigenetic chromatin marks, Molecular Pathway Enrichment and Mouse Knockout Gene Networks, the authors demonstrate an enrichment of regulatory elements in $CD4^+$ T-lymphocytes, and variation in genes expressed in T and B lymphocytes. These are fundamental parts of the immune system, and consolidate a model of the biological processes that are perturbed in RA. Secondly, the authors investigate polymorphisms in genes that are known drug targets of approved RA drugs. By annotating RA risk SNPs to nearby genes, and annotating approved RA drugs to genes, the authors demonstrate 3.7 fold enrichment for RA drug targets within RA risk genes. This approach is then extended to identify approved drugs for other disorders that may have an efficacy in the treatment of RA. Thus Okada et al demonstrate the utility of annotation and post-GWAS analyses to gain deeper insight into the biological pathways involved in in complex diseases and thus potential novel therapeutic approaches.

Before the advent of the GWAS era, a number of 'candidate genes' – genetic variation that would be expected to contribute to risk of disease based on our understanding of the biological systems involved – were identified that increase risk of autoimmune disorders. T-cells, which co-ordinate and effect cell death in the active immune response, learn self-tolerance through a process called Thymic Selection, mediated by a class of protein called Human Leukocyte Antigens (HLA). Many HLA proteins are expressed on

the wall of the thymus and effect the identification and removal of any T-cells that might react to an individual's own tissues. There are many different proteins that function in this way as HLA molecules, and variation in different genes has been associated with different autoimmune disorders. Rheumatoid Arthritis risk is increased by variation in the *HLA-DRB1* gene, the most significantly associated risk allele being *HLA-DRB1*04:01* (Raychaudhuri et al., 2012); this variant also increases risk of type 1 diabetes, as does the *HLA-DQA1*03:01* allele (Pociot & Lernmark, 2016; Sanjeevi et al., 1995). Variants in other genes coding proteins involved in T-cell activity have also been reliably associated with increased risk of autoimmune disease - such as *PTPN22*, which modulates the sensitivity of T-cells to thymic selection, and *CTLA4*, which codes a protein that modulates the activation of 'killer' T-cells by antigens identified as 'non-self'. In this way, understanding of the pathophysiology of a disorder can target the discovery of novel genetic associations, and similarly the understanding of novel genetic associations can contribute to understanding biological pathways perturbed in disease. Since the advent of GWAS, many candidate gene findings have been shown to be spurious, and so, generally, the hypothesis-free nature of GWAS and its stringent significance threshold is now preferred for identifying the genetic components to disease risk.

The genetics of psychiatric disorders

Five psychiatric disorders have been the target of intensive GWAS investigation in recent years– Attention Deficit Hyperactivity Disorder (ADHD), Autism, Bipolar Disorder (BPD), Major Depressive Disorder (MDD) and Schizophrenia (SCZ). We focus on the latter two of these in this thesis. A recent report by the World Health Organisation shows that MDD is the leading cause of disability worldwide (World Health

Organisation, 2012); this is due to its comparatively early age at onset, impairment to work and the limited success of existing therapies. MDD is highly heritable (McGuffin, Katz, Watkins, & Rutherford, 1996) – with estimates from twin heritability ranging from 0.48 to 0.75, based on varying assumptions about the population prevalence of MDD - and a substantial proportion of the heritability of MDD has been shown to result from the effect of common risk alleles (SNP heritability = 0.32, $P < 10^{-3}$), (Lubke et al., 2012). Given that these estimates are significantly above zero, identifying risk alleles or risk profiles involved in the onset of MDD is feasible, and given the damaging consequences of MDD, it is a pertinent research question. Furthermore, a multitude of physical disorders are not only comorbid with MDD, but their prognosis is drastically exacerbated by such a comorbidity – e.g. cardiovascular disease: (Elderon & Whooley, 2013; Garfield et al., 2014).

Whilst MDD is the leading cause of disability worldwide, SCZ is amongst the leading causes of expense by healthcare systems, families and governments (Knapp, 1997). Costs carried in the USA by SCZ include $63 Billion spent annually by families on treatment and lost due to time out of work (Wu et al., 2005). SCZ, like MDD, has an early age at onset and is frequently chronic across the lifespan. Like MDD, it is associated with increased healthcare utilisation - many autoimmune disorders are comorbid with SCZ (most recently discussed by Benros et al 2014), and SCZ patients are far more likely to engage in risky behaviour such as heavy tobacco (McCreadie & Kelly, 2000) and cannabis (Green, Young, & Kavanagh, 2005) abuse. Furthermore, SCZ is associated with increased involvement with the criminal justice system, both as defendant (Large & Nielssen, 2011; Richard-Devantoy, Orsat, Dumais, Turecki, & Jollant, 2014) and prosecution (Fitzgerald et al., 2005). Therefore improving our understanding of the

biological processes behind this disorder and disorders comorbid with it is important in the management of a number of outcomes.

The genetics of MDD

To date, only one published study has identified loci reaching genome-wide significance that affect risk of Major Depressive Disorder (CONVERGE Consortium, 2015). The CONVERGE study sought to increase power to detect loci increasing risk of MDD by obtaining a more homogenous subgroup of patients via deep phenotyping. Cases were recruited based on gender – female only – and severity – recurrent cases only; the authors hypothesised that this would identify a more heritable form of MDD in which the effect of risk variants would be larger and so a GWAS cohort might be well-powered to detect them. The authors find two genome-wide significant variants, one each in the genes *LHPP* and *SIRT1*. *LHPP* has been previously associated with MDD from linkage in family studies (Neff et al., 2009), and codes for a poorly understood protein involved in post-transcriptional modification (Kee & Muir, 2012). *SIRT1* is better understood, having a role in mitochondrial biogenesis, and may shed light on the pathogenesis of MDD. The authors of the CONVERGE study find that mitochondrial DNA levels are a predictor of number of stressful life events in a sample of MDD patients (Cai et al., 2015). Thus the 'hypothesis-free' nature of GWAS can identify previously unconsidered biological mechanisms involved in disease pathogenesis.

In addition to the results of the CONVERGE study, there are currently two unpublished studies that have identified loci reaching genome-wide significance in MDD. Power et al (in press) demonstrate that by obtaining deeper phenotype information on participants – in their case Age at Onset – it is possible to stratify participants and obtain a more homogeneous sample in which there is higher power to detect the genetic variants of small effect that one would expect to be involved in the pathophysiology of MDD

(Uher, 2009). The increase in power necessary to identify variants of small effect has also been achieved by the Psychiatric Genomics Consortium (PGC), who pool a large number of case-control cohorts investigating MDD (PGC-MDD in prep) and perform meta-analyses across these. As these two studies are currently unpublished, their results will not be discussed here in detail, other than to note that the small effect sizes that seem to underlie the effect of common genetic variation on risk of MDD requires a degree of innovation – such as relaxing the definition of MDD to self report cohorts to boost sample size in the case of PGC2, or stratifying based on age at onset to reduce genetic heterogeneity in the case of Power et al – in order to identify risk variants.

The genetics of schizophrenia

Genome-Wide Association Studies of schizophrenia have identified considerably more genetic risk variants than those in Major Depressive Disorder. The most recent Genome-Wide Association Study identified independent association signals at 128 loci across 108 genes; as the largest association study of a neuropsychiatric trait to date, the genetic architecture identified in the study of schizophrenia is likely illuminate the study of other psychiatric disorders (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Many novel loci associated with schizophrenia in this study are in genes consistent with historical drug targets in schizophrenia – for example *DRD2*, which codes a type 2 Dopamine receptor subunit, is a target of many antipsychotics, such as haloperidol. Other identified loci validate a neuropsychiatric aetiology to the pathogenesis of schizophrenia, with genome-wide significant loci in the voltage-gated Ca^{2+} channel subunit genes *CACNA1C*, *CACNB2* and *CACNA1I*, important for synaptic neurotransmission.

In addition to brain-expressed genes associated with schizophrenia, the PGC also identified variants suggesting an immune component to the aetiology of schizophrenia. Alongside identifying variants in brain-expressed genes, the authors identify a substantial enrichment of variants expressed in $CD20^{+}$ B-lymphocytes. An involvement of immune function in the aetiology of schizophrenia has been a prominent finding in association studies; the most significantly associated risk variants for schizophrenia are in the Major Histocompatability Complex (MHC) between 26 and 33 Mb on chromosome 6, a genomic region that contains a high concentration of immune-related proteins. This is consistent with models of the biology of schizophrenia, which will be discussed below.

Evidence for Genetic Overlap

At the heart of the idea of genetic overlap is the idea of *pleiotropy*. Whilst a protein may perform a restricted role within a pathway, any deformity in this protein may result in a number of different downstream effects. If this deformity is caused by genetic factors, and downstream effects include symptoms of different diseases, this can constitute pleiotropy.

Genetics of MDD and the immune system

There have been a wealth of 'candidate studies', investigating association between polymorphism at a given base pair position or gene and a phenotype. Despite the extensive literature, which often produced replicable findings, not one of the candidate genes for MDD replicates in the most recent and higher powered GWASs for MDD (CONVERGE Consortium, 2015; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013). It is unlikely that considering the candidate gene literature (Flint & Kendler, 2014) will contribute to our understanding of the

genetic architecture of MDD. Furthermore, we were unable to identify any family-based studies investigating an association between family history of an autoimmune disorder and MDD in probands; such studies have met with considerable success in SCZ research (Benros, Pedersen, et al., 2014), and so there may be merit in conducting this investigation in the future.

Genetics of SCZ and the Immune System

The most robust genetic association with schizophrenia is within the MHC region on chromosome 6p. This region is responsible for encoding a number of cellular receptors involved in antigen presentation, important in the T-cell system. Recently, however, Sekar et al have localised this signal to the C4 genes, polymorphisms in which are associated with differential cortical pruning and thus related to the organic abnormalities seen in the post mortem brains of schizophrenia patients (Sekar et al., 2016). The authors find that this association is unrelated to classical HLA class I and class II genes, and thus is unlikely to relate to autoimmunity. Despite this, GWAS has identified schizophrenia risk loci across the genome – not just in the MHC region – and so this finding does not eliminate the possibility for genetic variation involved in schizophrenia also having an influence on immune phenotypes. From statistical genetic studies, evidence for this comes from pathway-based analyses of loci associated with schizophrenia, bipolar disorder or major depressive disorder – phenotypes often grouped together as 'severe mental illness' (Uher, 2014) – pooled to increase power. Pooling these phenotypes and meta-analysing pathways associated with risk alleles, O'Dushlaine et al find an enrichment for loci involved in synaptic function – as would be expected from neuropsychiatric phenotypes – but also in immune pathways, a finding that the authors note warrants further exploration (Network Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015).

Furthermore, a family history of several heritable autoimmune disorders have been identified as risk factors for SCZ, including autoimmune thyrotoxicosis (Eaton et al., 2006), T1D (Gilvarry et al., 1996), multiple sclerosis (Eaton, Pedersen, Nielsen, & Mortensen, 2010), and a plethora of others as reviewed by Benros et al (Benros, Eaton, & Mortensen, 2014). Thus there is evidence to postulate that some of the same genetic variants may influence risk of both SCZ and also several autoimmune disorders – although it is possible that shared environmental risk factors may be captured by studies of family history, and is an important caveat when interpreting these findings.

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Introduction to Research Methods

Statistical Genetics

Complex disease genetics is the study of disorders with a genetic component to disease risk; crucially, this is comprised of many risk alleles, each of small effect. Alleles are measured as polymorphisms at single base pair positions, termed single nucleotide polymorphisms (SNPs). The most widely-used method to date for measuring an individual's genome-wide genetic variation is to use a genotyping chip, which genotypes – that is to say detects variation - at these SNPs. Widely used chips currently tag around 500,000 SNPs genome-wide. Each of these is typically biallelic - i.e. can take one of two alleles - and autosomal SNPs are diploid - that is to say for every genotyped locus on chromosomes 1 to 22, an individual has two copies of each locus, one on each chromosome in a pair. For economical reasons, genotyping chips usually only tag alleles with a minor allele frequency (MAF) in a control population above a given threshold, typically about 0.5 - 1%, and data is usually cleaned to restrict SNPs to a similar

threshold, depending on sample sizes and thus statistical power. Alleles with a MAF above the cut-off threshold for a given study can be termed 'common alleles'. A fundamental challenge in statistical genetics, therefore, is to determine association between a large number of often correlated SNPs, genome-wide, and some trait, given that each of these SNPs is likely to have a very small contribution to disease risk if any at all

Although the field of statistical genetics has a rich history before the advent of genome-wide genotyping arrays, a substantial proportion of statistical genetic research focuses on the analysis of SNP data. 2007 is often stated as the year that the analysis of genome-wide SNP data truly began to come into its own. The Wellcome Trust Case Control Consortium (WTCCC) published a landmark study on 7 complex diseases, using multiple univariate logistic regression to test association between ~500,000 genome-wide SNPs and each of 7 diseases (Wellcome Trust Case Control Consortium, 2007). This method was developed in the first successful GWAS (Klein et al., 2005), studying Age-related Macular Degeneration. The effect size of their risk allele, in the *CFH* gene, was large enough to detect in their sample of 146 individuals – an Odds Ratio of 7.4; most complex disease risk alleles have much smaller effect sizes – 1.1 to 1.3 - and so increasing the size of GWAS samples has proven a reliable way to improve the identification of risk alleles. There are also a number of statistical genetics techniques that have been developed to improve GWAS. Examples of this include Principal Component Analysis (PCA) and Imputation. PCA controls for heterogeneity between cases and controls, which might lead to identifying spurious associations (Price et al., 2006). Imputation leverages Markov-Chain Monte-Carlo methods to make a 'best guess' for missing genotypes - using this method, it is possible to genotype 500,000 variants directly but impute up to over 10 million variants, vastly improving resolution genome-wide

(Marchini, Howie, Myers, McVean, & Donnelly, 2007). Thus optimisation of the GWAS by novel methods and larger sample sizes demonstrate that this established technique has a future in identifying the genetic component to disease.

Many authors (Juran & Lazaridis, 2011) have claimed that we are now in the *post-GWAS* era - this leads to the second broad domain of statistical genetics and use of human SNP data. Aside from GWAS, there are a number of other analyses available on SNP data, many of which will be discussed below. Many post-GWAS methods leverage existing GWAS summary data in order to perform posterior analyses; other post-GWAS methods use SNP data to answer questions on the genetic architecture of a phenotype without ever performing an association study. Such techniques include Genome-Relatedness-Matrix Restricted Maximum Likelihood (GREML), (Yang, Lee, Goddard, & Visscher, 2011), which will be covered below. GREML builds a matrix of genetic similarity across a large sample of unrelated individuals and then fits a mixed model to predict the proportion of variance in phenotype explained by variance in genetic similarity. This is an approximation for heritability as estimated by twin studies. Twin studies dominated human genetics prior to DNA based methods such as GWAS and linkage - therefore calculating SNP-heritability generates results that are comparable in interpretation with historic estimates and has found substantial popularity in recent years.

Genome-wide association studies (GWAS) are limited to discovering common risk alleles, due to the coverage of most chips. Aside from this, the small effect sizes predicted in complex disease genetics mean that the primary obstacle in identifying all common variants associated with risk of a disease is one of power. That is to say, the likelihood of detecting an effect given the significance threshold used, the sample size used and the size of that effect. Small effect sizes require a large sample size in order to

detect them. Therefore, it could be argued that the primary objective for complex disease genetics is the collection of large enough samples in order to detect the small effect sizes underlying disease risk. This can be seen directly in GWAS of schizophrenia, where progressive increases in sample sizes from 2009 – 2014 have generated increasing numbers of novel GWAS findings, from 3,322 cases and 3,582 controls identifying zero regions significantly associated with schizophrenia in 2009 (International Schizophrenia Consortium et al., 2009), to 9,394 cases and 12,462 controls identifying 7 SNPs in 2011 (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011) and most recently 34,241 cases and 82,315 controls identifying 128 SNPs in 2014 (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014).

Whilst the identification of individual risk alleles at genome-wide significance is a power consideration requiring large sample sizes, there are many other statistical genetics techniques available for use with datasets that are underpowered. Even MDD datasets, which have yielded few SNPs reaching genome-wide significance to date, can be leveraged via a variety of methods in order to make meaningful inferences about the genetic architecture of MDD. Here we will outline methods that use genome-wide SNPs outside of a GWAS framework and discuss how they may be applied to dissect the genetic architecture of comorbid disorders, a central goal to our investigation of the psychiatric and autoimmune disorders.

Polygenic Risk Scoring

The functional unit of genetic epidemiology is the probability of an individual *i* of disease, given a number of predictors x. This is $Pr(y_i = 1 \mid x_i)$. Our task is to identify predictors, x_i, that have a genetic origin. This in turn illuminates the biology and epidemiology of a disease. Polygenic Risk Scoring, PRS, combines genotype data and *a*

priori information about how these variants associate with a given disease, to produce a single measure per individual that captures the probability of an individual having a disease conditional on their genetics - a genetic risk profile

The GWAS era has been fully fledged for almost a decade, and so a wide range of GWA studies' results are now in the public domain. It is a simple task to download a list of genome-wide SNPs and their association with a given phenotype - summarised by a normalised association statistic and effect size – i.e. *P*-value and either natural logarithm of Odds Ratio or regression coefficient. When a large number of SNPs reach genome-wide significance for a disease, we may construct a risk profile by summing an individual's risk allele count at each disease locus, each weighted by the effect size of the risk allele at this locus – this uses a method developed by Purcell et al (International Schizophrenia Consortium et al., 2009) and will be discussed in detail below.

A large number of loci are involved in risk of complex traits, however a relatively small number (tens to hundreds) have been discovered at genome-wide significance - $\alpha = 5 \times 10^{-8}$ (Dudbridge & Gusnanto, 2008; Panagiotou, Ioannidis, & Genome-Wide Significance Project, 2012) – for a given trait. This is due in part to statistical power. Formally, a *P*-value is the probability of seeing the observed data or anything more extreme, under the null hypothesis – i.e. by chance. Many authors have sought to exploit the fact that this is a continuous measure, and therefore SNPs that are associated with a phenotype with a low but non-significant *P*-value are more likely to have a reproducible and robust effect on outcome than a SNP with a higher *P*-value. Given this, all SNPs from a GWAS – termed *base* in the PRS literature - can be ordered by *P*-value and selected based on some *P*-value threshold, P_T. These can then be treated as if genome-wide significant to calculate a risk score using the protocol described above. We calculate risk scores in an

independent genotyped and phenotyped population – termed the *target* data - and calculate the prediction of risk score on phenotype at each threshold P_T. We select the SNPs at the threshold that best predicts phenotype in the target dataset - this is a *polygenic risk score*. This can be thought of as an optimum trade off between signal - disease SNPs rejected due to low power and stringent α level - and noise - SNPs with a low *P*-value by chance and no role in disease aetiology

A few methodological considerations are necessary when performing polygenic risk scoring (PRS). Firstly, as our risk model is additive on the log odds ratio scale, we will overestimate the effect of a particular risk variant if we include variants in high linkage disequilibrium (LD) with it. Therefore we clump SNPs in the base GWAS, using LD data as estimated in the target data, in order to obtain variants in approximate linkage equilibrium. For the same reason, authors frequently exclude the Major Histocompatibility Complex on Chromosome 6 entirely due to long range LD. As in a GWAS, it is necessary to adjust for population structure when testing the predictive value of polygenic risk score on disease status (Chen, Han, Hunter, Kraft, & Price, 2015); this can be performed using principal components. Finally, there are a number of methods for interpreting the predictive value of a PRS. The simplest is reporting the *P*-value of this variable from a multivariate logistic model controlling for population structure. The model fit can also be expressed as a measure of the total phenotypic variance explained – that is R^2 for continuous phenotypes, and Nagelkerke's Pseudo-R^2 - a coefficient of determination for logistic regression transformed to fall between zero and one – for case-control phenotypes.

Despite its development in 2009, the method of Polygenic Risk Scoring has only started to be widely used since the results of GWA studies have begun to be released publically

as the norm. This has led to a number of important papers applying PRS to illuminate our understanding of a number of behavioural phenotypes that had previously been hampered by power concerns. Power et al find that PRS for schizophrenia is a significant predictor of cannabis use, and arguing that these results support a model where a genetic predisposition to schizophrenia also predisposes individuals to try and use cannabis, rather than cannabis itself being a risk factor for schizophrenia – these findings may have important policy implications (Power et al., 2014). Secondly, Ruderfer et al use PRS for Schizophrenia to investigate the clinical dimensions amongst a cohort of bipolar disorder patients, finding that schizophrenia genetic risk significantly predicts manic symptoms but not negative, depressive or positive symptoms, thus illuminating the nature of the genetic overlap between schizophrenia and bipolar disorder (Ruderfer et al., 2014). Thirdly, a landmark study by Krapohl et al applied PRS for a large number of base GWASs to predict a large number of target phenotypes within the Twins Early Development Study (TEDS) data, with a focus on educational phenotypes (Krapohl et al., 2015). Although exploratory in nature, this study is important in that it represents a shift towards a more hypothesis-free approach to investigating the shared genetic relationships across multiple complex phenotypes. Thus, PRS can be used to dissect clinical heterogeneity in a sample, to infer causality to inform policy decisions and in an exploratory manner to identify patterns of genetic overlap within a well phenotyped cohort.

In addition to these applications of the PRS method, there has been considerable interest in interpreting the theoretical considerations – including power and the interpretation of phenotypic variance explained – by a number of authors (Dudbridge, 2013; Lee, Goddard, Wray, & Visscher, 2012). Power in polygenic risk scoring is an important consideration, and can be shown to be a function of a number of factors – the threshold

P_T used for PRS, the sample sizes of base and target samples, the *P*-value and variance explained for PRS on target phenotype, factors affecting ascertainment in base and target sample such as case control ratio and population disease prevalence, variance of marker effects in base and target sample and the number of markers used in PRS construction, and other factors which must be estimated and are not explicitly calculated when regressing PRS on phenotype, such as the covariance in marker effects between base and target sample, the proportion of the genome which is causal for a trait (typically denoted $1-\pi_0$).

The estimate for variance explained by PRS in a case-control target sample will not be immediately interpretable if the case-control ratio in the target sample is unequal to its ratio in the general population – i.e. if cases have been over-ascertained relative to the phenotype's population prevalence. Variance explained is dependant on the phenotypic variance – in the target sample, this is $nk(1-k)$ where n is the target sample size and k is the proportion of cases in the target sample. This will be maximised for fixed n when $k=0.5$, a typical case-control ratio in GWAS (Hong & Park, 2012). This artificial value for phenotypic variance in case-control studies gives an estimate of heritability that will not be immediately relatable to many epidemiological questions – heritability as estimated from a case-control cohort is termed heritability on the observed scale – often abbreviated to h^2_o. Heritability on the observed scale can be transformed to a more interpretable estimate, heritability on the liability scale – often abbreviated to h^2_l using a formula often referred to as the Robertson Transformation (Dempster & Lerner, 1950). This is $h^2_l = h^2_o k(1-k) / z^2$, where z is the normal density function evaluated at the truncation threshold – i.e. $\phi(t)$ - that differentiates cases and controls on a normally distributed but unobserved continuum of liability – a linear combination of genetic and environmental risk factors – and can be estimated from a phenotype's population

prevalence. Heritability on the liability scale illustrates variance explained in the general population, accounting for the lower prevalence and thus lower variance. Lee et al have provided formulae for the transformation of these estimates on the observed scale to the liability scale, based on estimates of prevalence and ascertainment, and more recently, methods such as ABC (Stahl et al., 2012) and AVENGEME (Palla & Dudbridge, 2015) allow PRS estimates to be compared to traditional heritability estimates. These transformations are valuable in the interpretation of PRS results and their dissemination.

Genomic Relatedness Matrix Restricted Maximum Likelihood

GREML is a two-step method developed by Yang et al that can approximate the heritability of a phenotype (Yang et al., 2011). In the first stage, a Genomic Relatedness Matrix (GRM) is calculated using pairwise genetic similarity across a large sample of individuals, using SNP data. Individuals with unusually high relatedness ($\hat{\pi} > 0.05$) are typically removed; these correspond to individuals who are closer relatives than 5th cousins, and so it is possible that the effects of shared environment could confound estimates.

The GRM can be used to fit a mixed model to calculate the proportion of phenotypic variance attributable to genetic similarity. It is usual to covary for population structure more stringently than in GWAS, using 20 principal components. Heritability is estimated in GREML using only 'common' SNPs - depending on the QC protocol used, this may be those with MAF >5% or similar. Therefore, the GREML estimate of heritability is heritability attributable to common genetic variation; these are the same variants that are under investigation in GWAS, so GREML heritability gives an estimate for the genetic architecture of a disorder that can be studied via GWAS.

Summary

Here we have demonstrated that there is substantial evidence for phenotypic correlation between many autoimmune disorders and two psychiatric disorders – schizophrenia and Major Depressive Disorder. We have presented evidence for immune-related pathways that seem involved in both Major Depressive Disorder (MDD) and schizophrenia (SCZ). These seem to segregate to being related to cytokines in MDD and antibodies in SCZ, although it is important to avoid binary distinctions in a field as interconnected as the mammalian immune system. SCZ genetics provides suggestive evidence for an autoimmune component to disease risk, however the same cannot be said of MDD, probably in part due to a lack of power (Flint & Kendler, 2014). Therefore our decision to use more sophisticated methods from statistical genetics to investigate the presence of shared risk factors seems well supported.

Outline To Thesis

In the following chapters, we investigate the questions posed above. Firstly, we explore the idea of 'genetic overlap' itself, in particular the method of Polygenic Risk Scoring discussed above, through the development of a novel software package and Polygenic Risk Scoring method, PRSice. We present this method alongside a more detailed explanation of the theoretical background to PRS and results from the application of PRSice, which is used throughout this thesis. We present three studies investigating the aetiological foundations of the overlap between the psychiatric and autoimmune disorders. Firstly, in a case-control study of rheumatoid arthritis, we investigate the role of schizophrenia genetic risk on rheumatoid arthritis status. Secondly, in a longitudinal population cohort, we use self report data on depression and autoimmune disorder onset

in order to compare the relative effects of genetic risk of depression and autoimmune disorder onset on subsequent hazard of depression – and the converse, the compare the relative effects of autoimmune disorder genetic risk and depression onset on subsequent hazard of developing an autoimmune disorder. Thirdly, within a clinical cohort of rheumatoid arthritis patients, we investigate the role of psychiatric symptoms, and depression genetic risk on the trajectory of patients' rheumatoid arthritis severity. Finally, alongside the future directions arising from the results of these three studies, we present the development of a novel method for calculating genetic risk of a phenotype by leveraging evidence for genetic overlap between traits.

Tables

Disorder	OR in SCZ Patients	95% CI
Any autoimmune Disease	1.53	1.46–1.62
Multiple sclerosis	1.57	1.29–1.90
Type 1 diabetes	2.83	2.58–3.10
Crohn's disease	1.33	1.08–1.61
Seropositive rheumatoid arthritis	0.75	0.60–0.93
Ulcerative colitis	0.99	0.84–1.16

Table 1: Epidemiological relationship between SCZ and a selection of autoimmune disorders (Benros, Pedersen, et al., 2014) – these phenotypes have been selected as autoimmune disorders which have been well-investigated by Genome-Wide Association Study, and so whose relationships may be investigated further by statistical genetics

Disorder	OR for MDD	95% CI	Study
Multiple sclerosis	2.3	1.6-3.3 *	Patten et al
Type 1 diabetes	2.9,	1.6–5.5	Anderson et al
Crohn's disease	1.67	1.31-2.09	Kurina et al
Seropositive rheumatoid arthritis	meta r = 0.21	$P < 0$ .0001	Dickens et al
Ulcerative colitis	2.39	1.54-3.53	Kurina et al

Table 2: Odds ratio for Major Depressive Disorder amongst autoimmune disorder patients relative to controls – i.e. a proxy for the overlap between depression and the autoimmune disorders in the same individuals, with values greater than one indicating an increased overlap than would be expected by chance. This approximates Relative Risk - a multiplier for Major Depressive Disorder prevalence amongst autoimmune disorder patients relative to controls - assuming a relatively low MDD prevalence around 10% (Viera, 2008).

* Odds ratio from multivariate logistic regression adjusted for age and sex

Autoantibody	***P*-value**
Dopamine Receptor	$P < 0.01$
GAD65	$P = 0.52$
Gastric Parietal Cell	$P = 0.42$
NMDA	$P < 0.01$
Rheumatoid Factor	$P < 0.01$
Serotonin	$P < 0.01$

Table 3: Proportion of positive autoantibody titres in SCZ cases and controls, from meta-analysis, (Ezeoke et al 2013)

Chapter 2: PRSice: Polygenic Risk Score software.

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Applications Note

OXFORD

Genome analysis

PRSice: Polygenic Risk Score software

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Abstract

Summary: A polygenic risk score (PRS) is a sum of trait-associated alleles across many genetic loci, typically weighted by effect sizes estimated from a genome-wide association study. The application of PRS has grown in recent years as their utility for detecting shared genetic aetiology among traits has become appreciated; PRS can also be used to establish the presence of a genetic signal in underpowered studies, to infer the genetic architecture of a trait, for screening in clinical trials, and can act as a biomarker for a phenotype. Here we present the first dedicated PRS software, PRSice ('precise'), for calculating, applying, evaluating and plotting the results of PRS. PRSice can calculate PRS at a large number of thresholds ("high resolution") to provide the best-fit PRS, as well as provide results calculated at broad *P*-value thresholds, can thin Single Nucleotide Polymorphisms (SNPs) according to linkage disequilibrium and *P*-value or use all SNPs, handles genotyped and imputed data, can calculate and incorporate ancestry-informative variables, and can apply PRS across multiple traits in a single run. We exemplify the use of PRSice via application to data on schizophrenia, major depressive disorder and smoking, illustrate the importance of identifying the best-fit PRS and estimate a *P*-value significance threshold for high-resolution PRS studies.
Availability and implementation: PRSice is written in R, including wrappers for bash data management scripts and PLINK-1.9 to minimize computational time. PRSice runs as a command-line program with a variety of user-options, and is freely available for download from http://PRSice.info
Contact: jack.euesden@kcl.ac.uk or paul.oreilly@kcl.ac.uk
Supplementary information: Supplementary data are available at *Bioinformatics* online.

1 Introduction

The polygenic model of human phenotypes has long been hypothesized, but only in recent years have the results from genome-wide association study (GWAS) revealed that much of the genetic basis for most complex traits comprises small effects of hundreds or even thousands of variants. For clinical outcomes, this polygenic effect can be considered a genetic liability to disease risk. While prediction of phenotype from an individual's genetic profile is compromised by this polygenicity, the application of polygenic risk scores (PRS) has shown that prediction is sufficiently accurate for a number of applications.

A PRS for an individual is a summation of their genotypes at variants genome-wide, weighted by effect sizes on a trait of interest. Effect sizes are typically estimated from published GWAS results, and only variants exceeding a *P*-value threshold, P_T, are included (Dudbridge, 2013). Since even large GWAS achieve only marginal evidence for association for many causal variants, PRS are usually calculated at a set of *P*-value thresholds, e.g. $P_T = 1 \times 10^{-5}, 1 \times 10^{-4}, \ldots, 0.05, 0.1, \ldots, 0.5$. A common application of PRS is to test for shared genetic aetiology between traits. Here PRS on the *base phenotype* are calculated, using GWAS results, in individuals from an independent data set, and these are used as predictors of the *target phenotype* in a regression (see Supplementary Note S1). This technique was first applied by the International Schizophrenia Consortium (2009), demonstrating that genetic risk for schizophrenia (SCZ) is a predictor of bipolar disorder. This study also acted as a proof-of-principle for PRS, showing that PRS based on thousands of common variants genome-wide, including many

with no effect and using effect size estimates from published GWAS, can provide a reliable indicator of genetic liability. This has motivated several other applications, including polygenic Mendelian Randomisation (Hung *et al.*, 2014), where causality of potential intermediate phenotypes in a disease pathway can be tested (Ehret *et al.*, 2011), use of PRS as biomarkers, and the enrolment of clinical trial participants according to risk (Hu *et al.*, 2013).

Here we describe the first dedicated and fully automated software package for the application of PRS - PRSice. PRSice has a high-resolution option that returns the best-fit PRS, has a flexible set of user options intended to capture current standard practices in PRS studies and the different applications of PRS, and produces plots for inspection of results. We also perform a simulation study to estimate a *P*-value significance threshold for high-resolution PRS studies.

2 Overview of PRSice

PRSice has been developed to fully automate PRS analyses, substantially expanding the capability of PLINK-1.9 (Chang *et al.*, 2014). A key feature of PRSice is that it can calculate PRS at any number of *P*-value thresholds (P_T) and can thus identify the most predictive (precise) threshold. It requires only GWAS results on a *base phenotype* and genotype data on a *target phenotype* as input (base and target phenotype may be the same); it outputs PRS for each individual and figures depicting the PRS model fit at a range of P_T. PRSice allows users to include or remove SNPs in linkage disequilibrium, handles genotyped and imputed data, and can calculate ancestry-informative dimensions for use as covariates. These features integrate R code with computations performed in PLINK-1.9 and extensive bash scripts to minimize computational time. PRSice is a command-line program that allows users to apply a large number of PRS, under different parameter settings or across multiple base and target traits. In addition to the standard approach, there is an option to use summary statistics for the target as well as the base data, using the gtx package (Johnson, 2013). For future updates of PRSice, see the website: http://PRSice.info.

3 Results

Here we illustrate the use of PRSice to test for shared genetic aetiology between traits. We first investigate the genetic relationship between schizophrenia (SCZ) and major depressive disorder (MDD), both known to be complex and comorbid. We apply PRSice to replicate the finding by Smoller *et al.* (2013) that SCZ PRS can predict MDD status, using the RADIANT-UK MDD case-control data set (Supplementary Note S2, Lewis *et al.*, 2010). Applying PRSice, we remove SNPs in linkage disequilibrium and include principal components to control for population structure. We find significant evidence that SCZ PRS predict MDD status, and under the approach of only testing PRS at several broad *P*-value thresholds find the most predictive threshold at $P_T = 0.05$ (Fig. 1). Next we repeat the analysis using high-resolution PRS (Supplementary Note S3) and find the most predictive PRS at $P_T = 0.0265$ (Fig. 2). The PRS at $P_T = 0.05$ explains 1.5% of the variation in MDD (Nagelkerke R^2; $P = 1.3 \times 10^{-9}$) whereas the high-resolution best-fit PRS explains 2.1% ($P = 2.1 \times 10^{-12}$) and is based on 5252 fewer SNPs (12148 rather than 17400).

Next we apply PRSice to two tobacco-related phenotypes from the TAG consortium (Thorgeirsson *et al.*, 2013) and the RADIANT-UK MDD data. These analyses reveal, for the first time, shared

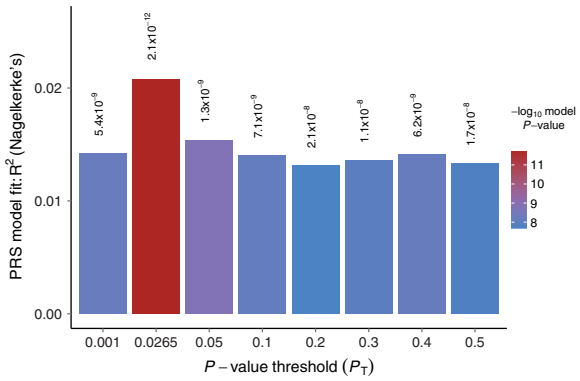

Fig. 1. Bar plot from PRSice showing results at broad *P*-value thresholds for Schizophrenia PRS predicting MDD status. A bar for the best-fit PRS from the high-resolution run is also included

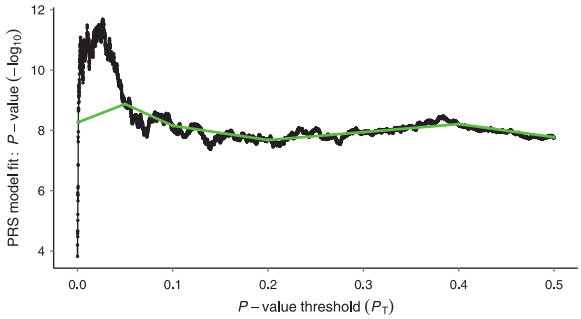

Fig. 2. High-resolution PRSice plot for SCZ predicting MDD status. The thick line connects points at the broad *P*-value thresholds of Fig.1

genetic aetiology between the dichotomous trait 'ever smoked' and MDD, but not between smoking consumption, as a quantitative trait, and MDD (Supplementary Fig. S1). In the former, high-resolution scoring again produces a substantially different best-fit PRS than that from broad P_T, in terms of model fit, significance and number of SNPs included (Supplementary Fig. S1b).

Under high-resolution PRS in particular, multiple tests are performed and so the *P*-value of the best-fit PRS will be inflated. Therefore, we perform a permutation study utilizing the SCZ and MDD data described above, and estimate an adjusted significance threshold for the best-fit PRS of $P = 0.004$ (Supplementary Note S4). Prior to a more extensive study, we suggest a more conservative significance threshold of $P = 0.001$ if using the best-fit PRS for association testing in PRS studies.

4 Discussion

Here we have described our PRSice software, illustrating its use with three PRS studies. We have illustrated the potential benefit of obtaining the best-fit PRS and have estimated a corresponding significance threshold. There is great potential for the future application of PRS in genetics: for gaining insights into the genetic architecture of a trait by comparing observed PRS with theoretical expectations across a range of P_T (International Schizophrenia Consortium, 2009), for assessing the genetic overlap of a trait(s) across populations, for use as biomarkers, as instrumental variables, or even to provide evolutionary insights (Berg and Coop, 2014). The PRS approach, and PRSice software, could be extended to test the effects of copy number variants, epigenetic markers and more. We believe

that PRSice can simplify PRS studies greatly, expand the application of PRS and aid the implementation of best-practice in PRS studies.

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Supplementary Material

PRSice: Polygenic Risk Score software

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Supplementary Note 1: Polygenic Risk Scores applied between traits

Polygenic risk scores are calculated across *n* individuals from the 'target phenotype' data set using a list of *m* SNPs, the genotypes of which have some effect (or not) on the 'base phenotype'. The base and target phenotype may be the same, if assessing the shared genetic overlap of a phenotype between samples/populations. These genotype effects can be estimated from a univariate regression of base phenotype on each SNP, such as from a genome-wide association study (GWAS). In such a GWAS, for a SNP *i*, where *i = 1, 2, ..., m*, a *P*-value, P_i, is calculated for the association between the SNP genotypes, $G_{i,j} = \{0,1,2\}$ for individual *j* where *j* = 1, 2, … , *n*, and the phenotype. Under the usual additive assumption made in GWAS, a corresponding effect size is estimated, by β_i, for the effect of a unit increase in genotype, G_{ij}, on the phenotype.

SNPs are generally selected for inclusion in a polygenic risk score based on the degree of evidence, according to *P*-value, for their association with the base phenotype in a GWAS – SNP *i* will be included in a PRS if P_i is smaller than a threshold, P_T. PRS are typically calculated at a number of different *P*-value thresholds, P_T.

At threshold P_T, the PRS for individual *j* can be calculated as:

$$PRS_{P_T,j} = \sum_{i=1}^{m} \beta_i G_{i,j}$$

PRS, which we see here are based on effect size estimates relating to the 'base phenotype', are calculated across all individuals giving *n* scores per threshold, P_T. The association between these PRS and the target phenotype can then be evaluated in an appropriate regression model (depending on the data type of the target phenotype, eg. linear regression if the phenotype is continuous).

This can be repeated across *q* *P*-value thresholds, P_T, and the model fit of the regression of target phenotype on PRS compared.

In real data there is usually some missing genotype data, unless genotypes have already been imputed. PLINK-2 imputes any missing data according to mean allele frequencies.

Supplementary Note 2: Data sets analysed

Base phenotype data set
In the main analysis we used the publicly available results from the largest Schizophrenia GWAS to date (Psychiatric Genomics Consortium (2014)) for the base phenotype data set. In the additional analyses on Smoking behaviour and MDD, we used GWAS results from the Tobacco and Genetics (TAG) consortium on two phenotypes as the base phenotype: the binary phenotype 'ever smoked' and the quantitative trait smoking consumption, as measured by average number of cigarettes smoked per day (Thorgeirsson et al. (2013)). In each case we removed any SNPs with poor imputation quality (info score < 0.7).

Target phenotype data set
We used genotype data from the RADIANT-UK consortium (Lewis et al. (2010)), a sample of 1624 depression cases and 1588 psychiatrically screened healthy controls, for the target phenotype data set for each PRS analysis. These were genotyped on the Illumina HumanHap 610 QuadBead Chip. Quality control was performed, removing individuals with missingness > 1%, abnormal heterozygosity, conflicting sex and reported gender and those of non-European ancestry or close relatedness based on principal components. SNPs with MAF < 1% and SNPs not in HWE ($P < 1x10^{-5}$) were also removed.

We used the first two eigenvectors calculated using EIGENSTRAT as ancestry informative dimensions, to adjust for population structure. Linkage disequilibrium (LD) was accounted for by selecting the SNP in the base phenotype data set with the lowest discovery *P*-value in a sliding window of 250kb, only retaining variants with a pairwise LD $r^2 < 0.1$, according to LD calculated in the target data set. We performed high-resolution scoring by testing every threshold between $P_T = 0.0001$ and $P_T = 0.5$ at increments of 0.00005. This produces 9999 thresholds.

Supplementary Note 3: High-resolution polygenic risk scoring

High-resolution polygenic risk scoring, as performed in PRSice, calculates PRS at a large number of evenly spaced *P*-value thresholds, between a minimum and maximum bound. For the analysis here, we use a lower bound of $P = 0.0001$ and an upper bound of $P = 0.5$, and increments of 0.00005. This generates 9999 thresholds. Assuming that there are ~100k SNPs in approximate linkage equilibrium with $P < 0.5$, 10 SNPs would be added per threshold if *P*-values were uniformly distributed across SNPs. In practice, GWAS results will be enriched for small *P*-values, due to association with the base phenotype and due to *P*-value informed clumping preferentially extracting SNPs with small *P*-values. Therefore, the number of SNPs included at each threshold will decrease at larger *P*-value thresholds. This high-resolution approach enables us to identify the best-fit PRS to a high degree of approximation; the true best-fit PRS can only be identified by testing PRS at every possible P_T, but we instead test them at high-resolution in order to reduce total computational time substantially with negligible loss in accuracy.

Supplementary Note 4: Multiple testing correction

High-resolution polygenic scoring fits a large number of regression models, as described above, and so the 'multiple testing problem' should be addressed when evaluating the significance of the best-fit PRS. Currently, uncorrected alpha thresholds of 0.05 are routinely used to assess the significance of PRS. Under high-resolution a small number of SNPs are added to the model at each new *P*-value threshold. Thus, the resulting PRS is likely to be very similar to the previous, especially once a large number of SNPs are already included, so there is high correlation between the multiple tests performed. Therefore, a simple Bonferroni correction or similar for the number of tests performed will produce an overly conservative adjustment for the multiple testing.

We performed three permutation studies to estimate an appropriate significance threshold that controls the family-wise error rate at 0.05 and accounts for the multiple tests performed in a high-resolution PRSice analysis. We calculated PRS repeatedly at high-resolution, using the GWAS results on Schizophrenia from the Psychiatric Genomics Consortium as base data and RADIANT-UK genotype data on MDD as target data (see Supplementary Note 2), under the null hypothesis of no association with the target phenotype by permuting case-control status in the MDD data set. We used data from chromosome 19, which should reflect genetic data across the genome, and permuted MDD case-control status in the RADIANT-UK data set 10000 times. As above, we performed clumping on the SNPs to remove the effects of SNPs in LD and adjusted for population structure with two principal components. In this way we obtained an empirical distribution for the *P*-value of the best-fit PRS. In order to understand the effect of sample size on this distribution, we repeated our permutation study in 1000, 2000 and 3000 individuals randomly sampled from the target data. These results indicated that an alpha threshold of 0.004 must be applied to high-resolution best-fit PRS in order to ensure a false-positive rate below 0.05 (table S1). Prior to an extensive study to estimate a more reliable significance threshold for high-resolution PRS, we suggest a more conservative significance threshold of $P = 0.001$.

Sample Size	**Empirical Significance Threshold**
1000	0.0042
2000	0.0042
3000	0.0046

Table S1: Empirical significance thresholds calculated from permutation, estimating the required significance threshold to interpret the results of high-resolution scoring, across different target data set sizes.

Supplementary Figure S1

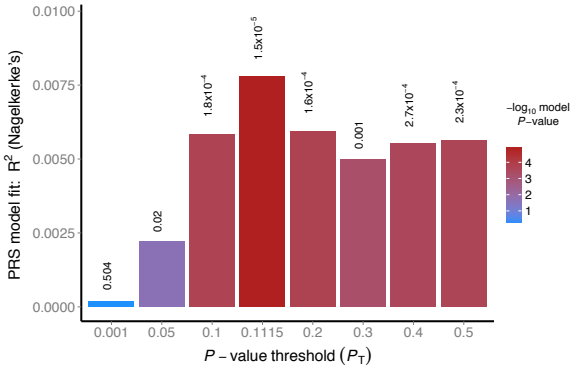

Figure S1a: 'Ever smoked': PRS using GWAS from the Tobacco and Genetics (TAG) consortium for 'ever smoked' as base phenotype data (N = 74053), and the RADIANT-UK MDD data as target phenotype data. SNPs in linkage equilibrium, adjusting for population structure using two principal components, show substantial evidence for shared genetic aetiology between smoking and MDD.

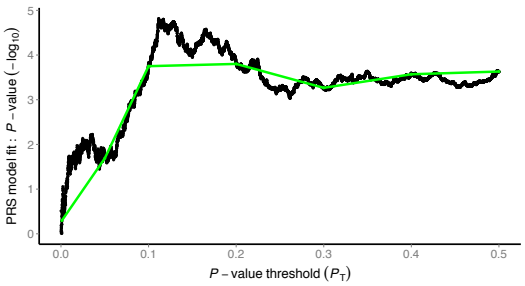

Figure S1b: 'Ever smoked': High-resolution PRS for 'ever smoked' predicting MDD status (see Fig. S1a). The high-resolution best-fit PRS is at $P_T = 0.1115$, while that based on broad thresholds on is $P_T = 0.2$.

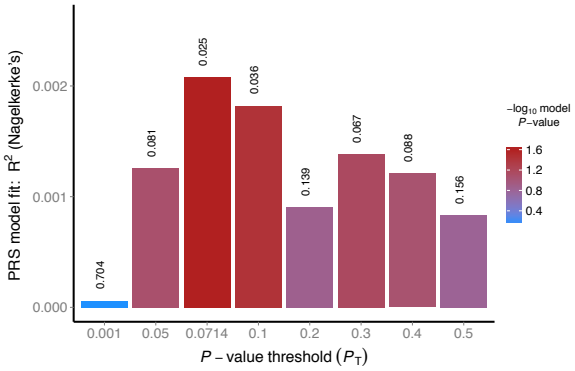

Figure S1c. Number of Cigarettes Smoked per day: Genetic risk of smoking more cigarettes as a quantitative trait, predicting MDD. This demonstrates no evidence for shared genetic aetiology between the two phenotypes, since the *P*-value of best-fit PRS (calculated from the high-resolution PRS) is > 0.001 (see Supp. Note 4).

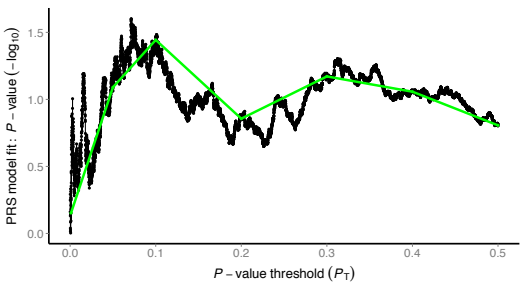

Figure S1d. Number of Cigarettes Smoked per day: High-resolution PRS for number of cigarettes smoked predicting MDD status. These high-resolution scores show that the results from the broad P-value thresholds of Figure S1c are not false negatives owing to the small number of thresholds considered.

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Chapter 3: The relationship between schizophrenia and rheumatoid arthritis revisited: genetic and epidemiological analyses.

RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART B
Neuropsychiatric Genetics

The Relationship Between Schizophrenia and Rheumatoid Arthritis Revisited: Genetic and Epidemiological Analyses

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Epidemiological studies are inconsistent on the relationship between schizophrenia (SCZ) and rheumatoid arthritis (RA). Several studies have shown that SCZ has a protective effect on RA, with RA occurring less frequently in SCZ cases than would be expected by chance, whilst other studies have failed to replicate this. We sought to test the hypothesis that this effect is due to a protective effect of SCZ risk alleles on RA onset. We first reviewed the literature on the comorbidity of RA and SCZ and performed a meta-analysis. We then used polygenic risk scoring in an RA case control study in order to investigate the contribution of SCZ risk alleles to RA risk. Meta-analysis across studies over the past half-century showed that prevalence of RA in SCZ cases was significantly reduced (OR = 0.48, 95% CI: 0.34–0.67, $P < 0.0001$). The relationship between SCZ genetic risk and RA status was weak. Polygenic risk of SCZ explained a small (0.1%) and non-significant ($P = 0.085$) proportion of variance in RA case control status. This relationship was nominally positive, with RA cases carrying more SCZ risk alleles than controls. The current findings do not support the assertion that the relationship between RA and SCZ is explained by genetic factors, which appear to have little or no effect. The protective effect of SCZ on RA may be due to environmental factors, such as an anti-inflammatory effect of anti-psychotic medication or merely due to confounding limitations in study designs.

Key words: Schizophrenia; Rheumatoid Arthritis; Comorbidity; Autoimmune

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INTRODUCTION

Rheumatoid arthritis (OMIM 180300) and schizophrenia (OMIM 181500) are, superficially, remarkably different disorders. They have similar prevalences; rheumatoid arthritis (RA) has an estimated point prevalence 0.6% [Helmick et al., 2008], whilst schizophrenia (SCZ) has an estimated point prevalence of 0.46% [Saha et al., 2005]. Lifetime prevalence for these disorders is substantially harder to measure, especially RA due to its later age at onset, however estimates for the lifetime prevalence of SCZ are as high as 0.72% [Saha et al., 2005]. Furthermore, both SCZ and RA show familial patterns of aggregation – heritability estimates for SCZ (0.81, 95% CI: 0.73–0.90) and RA (0.65, 95% CI: 0.50–0.77) are substantial [MacGregor et al., 2000]; [Sullivan et al., 2003]. This implies a complex genetic aetiology, in which many risk alleles of small effect size can aggregate in individuals to modulate their risk of developing a disorder. Alongside its familial pattern of aggregation, schizophrenia also shows an unusual aggregation of

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Abbreviations: SNP, Single Nucleotide Polymorphism; LD, Linkage Disequilibrium; MHC, Major Histocompatability Complex; HLA, Human Leukocyte Antigen; RA, Rheumatoid Arthritis; SCZ, Schizophrenia; GWAS, Genome-wide Association Study; PRS, Polygenic Risk Scoring; PGC, Psychiatric Genomics Consortium; WTCCC, Wellcome Trust Case-Control Consortium.
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comorbidities with many autoimmune disorders, such as Sjögren's Syndrome (OMIM %270150) [Eaton et al., 2006].

The relationship between SCZ and RA is much less clear, with many studies finding no evidence of a significant association [Eaton et al., 2006]. Here we review the findings of such studies in order to evaluate the veracity of this relationship. RA seems to be protective for SCZ, with studies reporting an OR for RA status in schizophrenia patients as low as 0.44 (95% CI 0.24–0.81). This suggests a substantial protective effect of the disorder [Mors et al., 1999]. This may be due to some risk factor for RA reducing schizophrenia risk, or vice versa. In order to understand this better, we apply a statistical genetics technique – polygenic risk scoring – to dissect the genetic relationship between the two disorders.

We are interested in explaining this relationship on three levels. On a genetic level, we are interested in the predetermined risk profiles carried by various individuals throughout their lifetimes; specifically the variance in disease status explainable by an individual's risk allele count. Secondly, we are interested in an epidemiological perspective – to explain the pattern of disease status and onset amongst a population, via a meta-analysis of studies investigating this. Finally we are interested in an aetiological perspective – the interaction between pre-existing risk and modulating factors that act to precipitate disease onset; we will examine aetiological and genetic data in order to make inferences on the aetiology of these two disorders.

RA is a joint disorder characterized by an elevation in levels of immune activity (e.g. increased T-cell proliferation) accompanied by painful, swollen, and ultimately, eroded and fused joints. Converging evidence from pharmacology, serology and genetics suggests that RA is an autoimmune disease. Its relatively high prevalence has made RA amenable to high throughput genetic studies, leading to the identification of, to date, 101 risk loci [Okada et al., 2014], providing invaluable clues to its aetiology. The strongest association for RA is in the Human Leukocyte Antigen (HLA) region. The HLA genes are located in the MHC region, on the short arm of chromosome 6 [Shiina et al., 2006], and are involved in adaptive immune response.

Schizophrenia is a psychiatric disorder, characterized by auditory hallucinations, delusions and disorganized speech. Historically, theories of psychiatric aetiology have been rooted in a Cartesian dichotomy, with disorders of the 'mind' predicted to have limited physiological aetiology or phenomenology [Kendler, 2012]. This has led to a number of environmental aetiologies proposed for schizophrenia – for example an environment with a high level of expressed emotion [Bebbington & Kuipers, 1994]. Despite this, there have been a number of studies arguing for an immune component to the aetiology of schizophrenia – this began with McGuffin et al 1978, based on serological studies. More recently, genome-wide association studies (GWAS) have identified genetic markers showing a significant association with schizophrenia. These genetic markers, Single Nucleotide Polymorphisms (SNPs) are studied across the genome in order to fine-map regions associated with disease and subsequently predict disease risk in other cohorts. Most robust amongst these associations is a region in the HLA, which shows strong association in all studies (Ripke, 2011; S.[Ripke et al., 2013].

Summary

We therefore sought to examine evidence for an epidemiological link between SCZ and RA by meta-analysis of studies investigating RA amongst SCZ patients. Given the polygenic architecture of these two disorders, we also investigated whether the genetic predictors influencing SCZ risk had an atypical distribution amongst RA patients.

RESULTS

Meta-Analysis

After following a protocol specified below, we identified 10 studies reported in 9 papers reporting the prevalence of rheumatoid arthritis

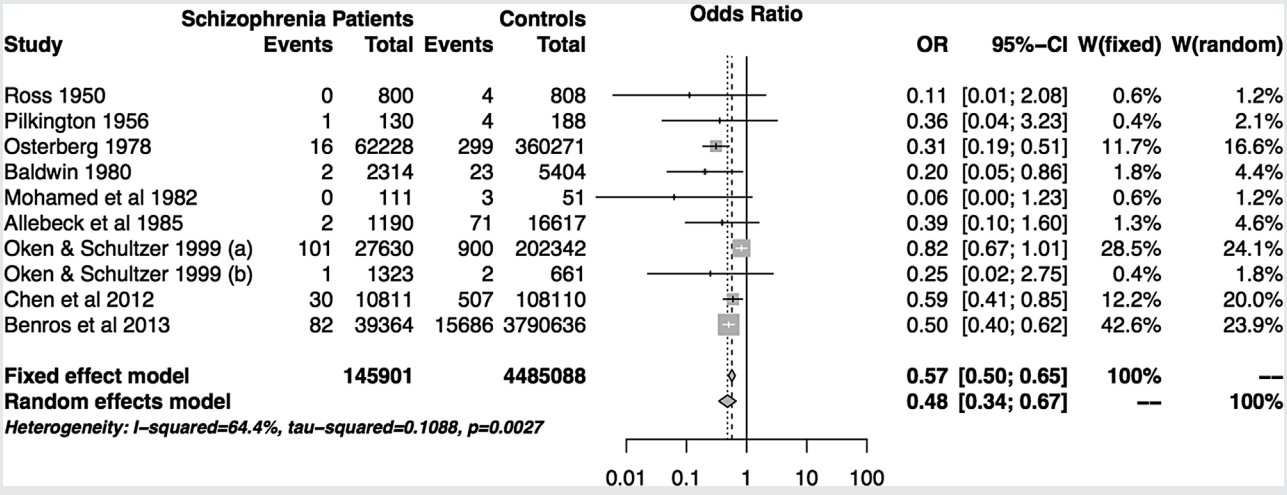

FIG. 1. Meta-analysis results. We identified 10 studies reported in 9 papers. Oken & Schultzer (a)compares schizophrenia vs other psychiatric patients in Canada meanwhile Oken & Schultzer (b) compares asimilar sample in New York State. We present the RA prevalence (events) in SCZ cases vs controls acrossstudies. W: weight for each study under random and fixed effects analysis.

(RA) within a schizophrenia (SCZ) sample and a sample of controls. We used the results of these studies to perform a meta-analysis (Fig. 1). Under a fixed effects model, SCZ status conferred an odds ratio of 0.57 (95% CI: 0.50–0.65, $P < 0.0001$) on RA status, and an odds ratio of 0.48 (95% CI: 0.34–0.67, $P < 0.0001$) under a random effects model, showing a significant protective effect of SCZ on RA status. There is statistically significant heterogeneity between studies ($P = 0.0027$) and therefore a random effects model is the most appropriate analysis approach.

These studies varied in their selection of controls – population controls, non-schizophrenic psychiatric patient controls and non-schizophrenic medical patients, and the most recent in a series of studies on a Danish population register comparing SCZ patients with population controls [Benros et al., 2013]. To maximise comparability, a major challenge in all epidemiological work, a number of studies use non-schizophrenic psychiatric patients as controls. This allows the effect of schizophrenia to be studied in isolation. Five of these studies are based in individual psychiatric hospitals - [Mohamed et al., 1982] Mohamed et al., 1982; [Ross et al., 1950]; [Pilkington, 1956]; [Oken & Schulzer, 1999] Ross et al., 1950). Two use record-linkage methods [Baldwin, 1980]; [Osterberg, 1978] on international and Swedish populations respectively. Finally two studies used general medical disorder patients as controls [Allebeck et al., 1985]; [Chen et al., 2012].

All studies estimated nominally lower risks of RA in SCZ cases compared to controls, and this relationship was statistically significant in four studies [Baldwin, 1980]; [Chen et al., 2012]; [Benros et al., 2013]; [Osterberg, 1978], replicating the canonical 'protective' effect of SCZ on RA. It is notable that these four studies are the largest included, all using record linkage databases and thus the remaining 6 studies, which failed to find any significant effect, may have been simply under-powered.

Polygenic Risk Scoring

We used published SCZ GWAS results (S.[Ripke et al., 2013] to calculate polygenic risk scores (PRS) in 1,989 RA cases and 1,588 controls. We used a series of thresholds, p_T, to select SCZ risk alleles based on GWAS p-value, and calculated risk scores for each of these risk allele sets (Table 1, Fig. 2a). SNPs associated with SCZ at $p_T < 0.01$ explain under 0.2% of the variance in RA status in the independent test cohort (Fig. 2b). This relationship is not statistically significant ($p = 0.085$) and is therefore no stronger than would be expected by chance. This is consistent with results using a considerably smaller SCZ sample (3,322 cases, 3,587 controls) as a discovery dataset [International Schizophrenia Consortium et al., 2009]. Standardised polygenic risk scores for SCZ at $p_T < 0.01$ are approximately normally distributed, with no significant difference ($p = 0.063$) in mean score between cases (0.028) and controls (-0.035), (Fig. 2c).

Genetic Profile Risk Scoring

We calculated a measure of SCZ genetic risk in our RA cases and controls using the panel of SNPs proposed by Ayalew et al, identifying proxies where necessary using SNAP [Johnson et al., 2008; Ayalew et al., 2012]. After QC, we obtained genotypes, imputed genotypes or proxies for 257 SNPs. SCZ genetic risk did not predict RA status – after adjusting for population structure, $P = 0.858$. We further explored the relationship between this panel of SNPs and RA, using SNP-based and gene-based summary statistic analyses (supplementary 8), and demonstrate that they do not show significant association with RA –SNP-based P-value = 0.13, gene-based P-value = 0.604.

Direction of Effect

We compared the direction of effect of risk alleles for SCZ and RA using published GWAS results for each [Stahl et al., 2010; Ripke et al., 2013]. After merging, 170,998 and 171,707 SNPs remained when clumping by RA P-value and SCZ P-value respectively, in order to obtain SNPs in approximate linkage equilibrium. The lack of association between SCZ alleles and RA alleles was confirmed through direction of effect analysis. We found no evidence for the proportion of alleles with a shared direction of effect between RA and SCZ deviating from our expectation under the null (Table II), using a Sign Test.

DISCUSSION

Our SCZ polygenic risk scores analysis has shown that variance in RA status cannot be predicted or explained by burden of SCZ risk alleles genome-wide. This is supported by an analysis of Genetic-Profile Risk Scores. On considering the epidemiology of these two disorders, this finding is consistent with the notion that there is no

TABLE I. Polygenic risk Scores for SCZ across thresholds and variance in RA status explained

Threshold, pT	Number of SNPs	Variance in RA status Explained, Pseudo R^2	P-Value
0.0001	82	0.0001	0.563
0.001	299	0.0004	0.276
0.01	1,393	0.0010	0.085
0.05	4,451	0.0007	0.154
0.1	7,396	0.0000	0.799
0.2	12,431	0.0000	0.816
0.3	16,708	0.0000	0.863
0.4	20,634	0.0000	0.770
0.5	24,122	0.0000	0.751

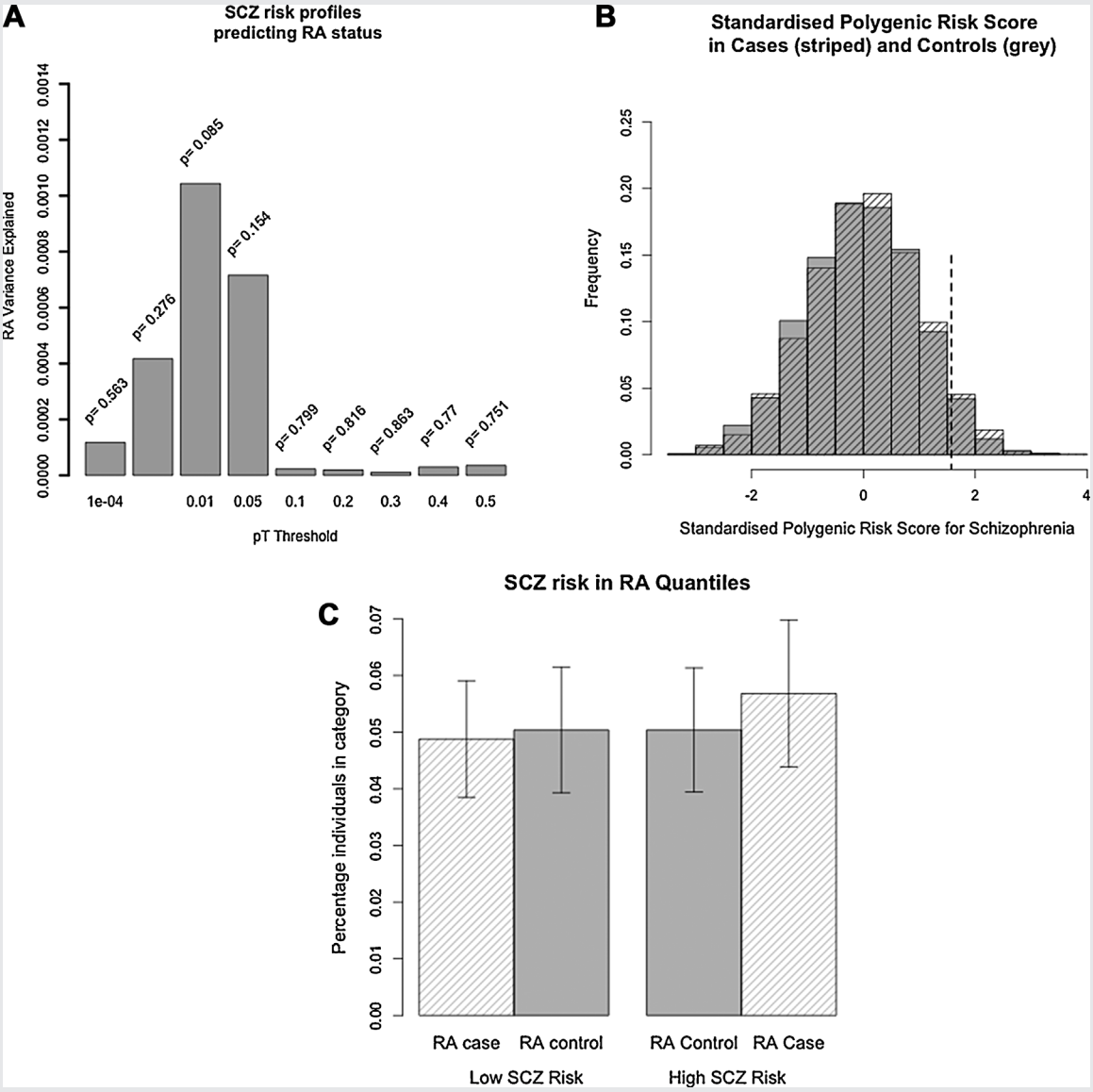

FIG. 2. (a)Variance in RA status explained by SCZ polygenic risk scores in an independent test cohort.Scores are calculated across cutoff thresholds, pT. (b) Standardised polygenic risk score distribution at pT $<$ 0.01 in RA cases (striped) and RA controls(grey). Dotted line - top quantile (highest 5%) for SCZ risk amongst controls (standardised score $>$ 1.57). 5.1% cases and 5.0% controls above this value. (c) SCZ risk in highest quantile (top 5%) and lowest quantile (bottom 5%) for SCZ risk between RA cases and controls.

'protective' effect of SCZ on RA – one would not be predicted from genetic data alone. Despite this, we have also demonstrated through meta-analysis that the negative association between the two disorders appears consistent across studies. Below we review the epidemiological and genetic evidence presented above, and propose some aetiological theories to reconcile them.

Epidemiology

The protective effect of SCZ on RA is supported in our meta-analysis, with an infrequency of RA in SCZ cases, which would not be predicted by chance. The possible protective effect of institutionalisation on RA status can be parsed from the effect of SCZ by looking at studies using institutionalised controls. Rothermich & Philips studied a prison population in order to investigate the protective effect of long term institutionalisation; although they found no significant relationship between RA and SCZ when using RA in prisoners as a control population, they found nominal, but non-significant, evidence of a protective effect of SCZ on RA onset [Rothermich & Philips, 1963]. This is consistent with equally underpowered studies using general psychiatric samples as controls.

Genetics

Both SCZ and RA have been associated with a number of risk alleles at genome-wide significance. Converging evidence for a lack of

TABLE II. Direction of Effect Sharing for SNPs in Approximate Linkage Equilibrium, Between SCZ and RA GWAS's

Clumped By	Threshold, p <	N SNPs	P, Pearson's χ^2	Proportion SNPs in same direction
RA	0.01	5,063	0.670	0.492
	0.1	35,795	0.569	0.503
	0.2	61,785	0.238	0.502
	0.3	83,174	0.241	0.502
	0.4	101,328	0.199	0.503
	0.5	117,664	0.152	0.503
SCZ	0.01	1,784	0.845	0.484
	0.1	16,503	0.966	0.496
	0.2	33,388	0.200	0.499
	0.3	50,159	0.217	0.498
	0.4	67,313	0.285	0.498
	0.5	84,613	0.179	0.498

shared genetic substrate between RA and SCZ comes from family studies of the two disorders [Benros et al., 2013]. The authors explored family history of SCZ (a proxy for SCZ risk allele burden) as a predictor of RA, finding no evidence of a significant association – the relative risk for family history of SCZ on RA risk was 0.94 (95% CI: 0.84–1.06).

Aetiology

The aetiologies of both rheumatoid arthritis and schizophrenia are still topics of active research. Evidence for an autoimmune substrate to schizophrenia has coalesced in recent years, driven by the genome-wide significant loci in the Major Histocompatibility Complex [Ripke., 2011; S.[Ripke et al., 2013]. This complements work in serological analysis of SCZ patients, notably [McGuffin et al., 1978], who found an increase in HLA-BW5 and a decrease in HLA-AW29 and HLA-BW17 in serum of SCZ patients (McGuffin, Farmer, & Rajah, 1978).

The association between SCZ and immune-related biomarkers may be reconciled as autoimmune over-activity specific to a component of the nervous system. A systematic review of blood protein expression in SCZ patients found evidence of increased autoantibodies for the N-methyl-D-Aspartate receptor (NMDA-R) [Ezeoke et al., 2013], which underlies the formation of associative memory by mediating the strengthening of synapses [Bannerman et al., 1995]. An autoimmune pathology could therefore underlie damage to neural tissue, and therefore networks, resulting in the cognitive symptoms observed in schizophrenia [van den Heuvel et al., 2013].

Despite the plausibility of this model, we do not find evidence for a genetic overlap between SCZ and RA. This suggests that, if some of the biological pathways involved in RA and SCZ are shared, it may be environmental rather than genetic aberrations perturbing these. Negative results must always be viewed cautiously in the context of power, and we discuss this limitation below.

Effect of Medication

We considered the epidemiological data on SCZ and RA in the light of their respective ages at onset. SCZ has a mean age at onset of around 26 years (95% CI 14.34 – 38.14) [Sham et al., 1994]. By contrast, RA has a much later age at onset, with the peak age at onset between 65–75 in men and 55–64 in women [Symmons and Deborah, 2002]. We considered that, by age at onset for RA, SCZ patients were likely to be medicated. If these two disorders do share an aetiological basis, antipsychotic medication may have a prophylactic effect on RA onset later in life.

The epidemiological studies presented above, exploring the relationship between SCZ and RA, do not stratify patients by medication status. It is unlikely, however, that medication status mediates the negative association. Chlorpromazine was first introduced clinically in the early 1950's, and clinical uptake of antipsychotics in the USA was gradual from the mid-1950's to the mid-1970 s [Shen, 1999]. Despite this, there is substantial evidence that typical antipsychotics such as haloperidol may have an anti-inflammatory role that may protect against RA. Synovitis and CRP levels in RA patients has been observed to improve following administration of haloperidol for acute mania in case studies, and in blood cultures stimulated acute inflammation led to a marked inhibition of the release of TNF α and IL1-β [Moots et al., 1999]. These inflammatory cytokines have been directly linked to RA [Elliott et al., 1995; McNiff et al., 1995]. Thus schizophrenia patients taking haloperidol may be protected from RA onset by the suppression of TNF-α and IL1-β levels.

Limitations

We identify four main limitations in our study. Firstly, as presented above, SCZ and RA have substantially different ages at onset, and the former is associated with substantially reduced life expectancy [Crump et al., 2013]; thus many SCZ patients may die before age at onset for RA. Many epidemiological studies above are unable to adjust for age amongst SCZ patients — in a record linkage paradigm, this data is not collected — and therefore we present unadjusted odds ratios for all studies. A 'harvesting effect' may confound the negative association between RA and SCZ [Sawchuk et al., 2013]; this is unlikely to account for the entire effect, as individual population registry studies, which collect sufficient data with sufficient power, replicate the negative association after adjusting for age [Benros et al.,

2013]. Furthermore, work on the Swedish Population Register has replicated the protective effect of SCZ on subsequent RA diagnosis using Cox regression models and adjusting for age (Hazard Ratio = 0.69, 95% CI = 0.59–0.80) [Sellgren et al., 2014].

Our RA cases and controls present a second limitation. They are genotyped on different microarrays, so we can only use SNPs shared across both platforms for calculating SCZ polygenic risk scores. As polygenic risk scoring requires SNPs in approximate linkage equilibrium, the number of SNPs remaining in our test dataset for polygenic scoring is similar to what would be expected when using a sample genotyped on a single platform. Although our controls have been screened for Major Depressive Disorder (MDD, OMIM 608516) and are not a true population cohort, GWAS have been consistently shown to be underpowered to detect risk variants associated with MDD [MDD Working Group of the Psychiatric Genomics Consortium et al., 2013] and so this is unlikely to affect our results.

Power considerations are a persistent concern in polygenic risk scoring. Calculation of power requires a series of assumptions to be made on the underlying architecture of the diseases studied, such as the correlation between genetic effects in the discovery and test datasets. Power calculations (Supplementary 6) show it is likely that we would have sufficient power to detect an epidemiologically meaningful correlation in genetic effects – assuming genetic effects at 1% of SNPs, we have 80% power to detect a modest genetic effect correlation (magnitude = 0.078) at $\alpha = 0.05$. Nevertheless, the possibility that our polygenic scoring results are a false negative is an important caveat.

Finally, as discussed above, RA and SCZ risk are both modulated by genotype at HLA loci. We have modelled this influence to an extent by including the most strongly associated SCZ risk SNP in this region in the calculation of polygenic risk scores. We estimated that SCZ status is protective for RA status with OR = 0.48 (95% CI: 0.34–0.67, $P < 0.0001$). The effect sizes of risk alleles in complex disease genetics are substantially smaller than this - the most significant MHC association with SCZ has an OR of 1.21 [Ripke et al., 2013]. Therefore it is unlikely that SCZ risk at the MHC alone could mediate the epidemiological effect calculated in meta-analysis above

Summary

Despite the mounting evidence for an autoimmune aetiology in schizophrenia, and epidemiological literature on the co-occurrence of these two disorders, we found no evidence for a shared genetic substrate between rheumatoid arthritis and schizophrenia, although this could be due to lack of power in the current samples. Epidemiological data may be confounded due to some protective effect acting to prevent onset of RA in high-risk individuals.

MATERIALS AND METHODS

Meta-analysis

We performed a systematic review and meta-analysis of studies investigating the prevalence of RA within SCZ patients. This was performed by searching Embase and Medline for articles published between 1945 and November 2013 containing the terms schiz$ AND rheuma$. We included only studies collecting data on RA within SCZ cases and a sample of SCZ controls. We restricted this to studies using population samples, non-schizophrenic psychiatric patients or other physical disorder patients.

We included all Journal Articles and retained Reviews meeting these criteria. We then read the bibliographies of all reviews and included any articles with relevant abstracts. Finally we read all articles extracted and retained those containing epidemiological studies of RA and SCZ prevalences, which also reported RA prevalences for SCZ controls. We extracted the following data; study name, authorship and year, case and control sample size, RA incidence in each of these populations and selection criteria for controls.

We excluded case studies and studies that did not also collect controls (see S3 for full details of method used). Literature search, data extraction and quality assessment was performed in an unblinded manner by J.E. We combined studies and calculated meta-analysis odds ratios under random effects and fixed effects using the R package meta.

Genetic Data Used

As a SCZ discovery data set, we used the most recent publically available results of GWAS of schizophrenia from a meta-analysis of the PGC1-SCZ study and a Swedish cohort (S.[Ripke et al., 2013], (full details of cohort in S5). This reported the p-value, odds ratio and test statistics for 9,898,079 SNPs imputed to the 1000 Genomes project [Siva, 2008]. For the RA target study, we used RA cases from the WTCCC study and controls from the RADIANT depression study. These controls were not included in the discovery study and therefore our discovery and test datasets are independent, as required for polygenic risk scoring. This contained data on 1,999 cases and 1,588 controls.

The WTCCC RA cases (n = 1,999) were collected across multiple UK studies co-ordinated by the Arthritis Research Campaign's Epidemiology Unit [Wellcome Trust Case Control Consortium., 2007]. All cases satisfied the criteria for RA specified by the American College of Rheumatology [Arnett et al., 1988].

The 1588 RADIANT controls were collected from the staff and student body of King's College London or recruited via the Medical Research Council's general practice research framework [Lewis et al., 2010]. They were screened negative for a lifetime history of any psychiatric diagnosis, using a modified version of the Past History Schedule (P. McGuffin, Katz, & Aldrich, 1986) and all reported to be of white European ancestry.

Cleaning Test Dataset

The RADIANT and WTCCC samples were genotyped on separate platforms (Illumina 610 quad bead and Affymetrix 500 k respectively), leading to a degree of attrition when merging datasets; after merging, 70,130 SNPs remained. We performed detailed quality control on the merged RADIANT-WTCCC dataset. The final data set contained 1,989 RA cases and 1,588 controls (table S1) with genotype data on 69,621 SNPs.

Cleaning Discovery Dataset

In the PGC and Swedish combined schizophrenia GWAS results as a discovery dataset, we removed SNPs with an info score less than 0.7,

indicating poor imputation quality and SNPs not present in the cleaned test dataset. Finally, in order to obtain SNPs in approximate linkage equilibrium, the HLA region (26–33 Mb on chromosome 6) was omitted, except for most significant SNP in this region (rs2517611). We used *P*-value-informed clumping, extracting SNPs based on linkage disequilibrium (LD) in HapMap2 CEU samples. This left 24,126 independent SNPs in our discovery data set.

Polygenic Risk Scoring

Polygenic risk scoring was performed in the RA test data set, based on SNPs extracted from the SCZ discovery data set meeting *P*-value thresholds p_T of 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5. At each threshold, p_T, SNPs with SCZ association *P*-values below the threshold were used to construct polygenic risk scores (PRS) for each individual in the RA test data set by summing the number of risk alleles at each SNP weighted by the natural logarithm of its odds ratio.

We then tested whether the SCZ PRS predicted variance in RA disease state in a logistic model, regressing disease state on PRS plus five ancestry-informative dimensions accounting for population structure. The variance in disease state explained by this model was calculated as Nagelkerke's pseudo R^2 (NR^2). We report the difference in NR^2 between this model and a model based on the ancestry-informative dimensions alone.

Genetic Profile Risk Scoring

We used the panel of 542 SNPs reported by Ayalew et al, which have been previously shown to serve as reliable predictors of SCZ status within independent cohorts and cohorts of different ethnicity [Ayalew et al., 2012], in order to construct genetic profile risk scores. We imputed our cases and controls to 1000Genomes and performed stringent QC. Of the 542 SNPs listed by Ayalew et al, we obtained genotypes or proxies with $R^2 > 0.6$ for 257. We calculated weighted scores for SCZ genetic risk in our RA cases and controls using these SNPs and the effect sizes reported by Ayalew et al, and fitted a logistic regression model adjusting for population structure using 5 ancestry informative dimensions calculated on genotyped SNPs.

Direction of Effect

In order to assess for consistency of direction of effect for SNPs between two schizophrenia and rheumatoid arthritis, we used published GWAS data from each disorder [Stahl et al., 2010]; [Ripke et al., 2013]. We performed quality control for imputation quality (as outlined above) and used *P*-value informed LD clumping to obtain relatively independent SNPs, using the same protocol above. For each clumped GWAS, we merged with GWAS results for the other disorder, extracted all SNPs below a particular *P*-value threshold, and classified SNPs as having the same direction of effect (both ORs > 1 for the same SNP allele), or different direction of effect. Consistency of SNP effect was tested for using Pearson's χ^2 statistics, commonly termed a 'sign test'.

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This study makes use of data generated by the Wellcome Trust Case-Control Consortium. A full list of the investigators who contributed to the generation of the data is available from *www.wtccc.org.uk*. Funding for the project was provided by the Wellcome Trust under award 076113 and 085475.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Supplementary Materials:

Supplementary 1: Cleaning Test dataset

Firstly, in order to account for any possible issues arising from using data from two different platforms, we analysed the WTCCC control dataset, genotyped on the Affymetrix 500k (3004 individuals, genotyped by the WTCCC, drawn from UK blood donors and the 1958 birth cohort, (Wellcome Trust Case Control Consortium, 2007)). We merged this dataset with the RADIANT controls, tested for association between the two data sets and removed any SNPs with $P < 10^{-5}$ for association from subsequent analyses. We also removed any SNPs with different physical positions between the two platforms, any SNPs with MAF < 0.05 in either control group, any SNPs with a difference in frequency > 0.15 between the two groups and any SNPs failing our general QC criteria outlined below. We removed any SNPs with genotyping rate < 0.99 or a *P*-value for Hardy-Weinberg Equilibrium $P < 5.7 \times 10^{-7}$. We also removed individuals with missingness > 0.03. This left 69,623 SNPs. Finally, we removed 10 SNPs reaching genome-wide significance ($P < 5.7 \times 10^{-8}$ which were not within 1 Mb of previously reported genome-wide significant loci for rheumatoid arthritis (Eyre et al., 2012), leaving 69,613 SNPs. We tested for cryptic relatedness, however no pairs of individuals met our exclusion criteria ($\hat{\pi} > 0.2$). The genomic control λ value between controls on the two chips to 1.097 indicating good consistency (**fig S1.a**).

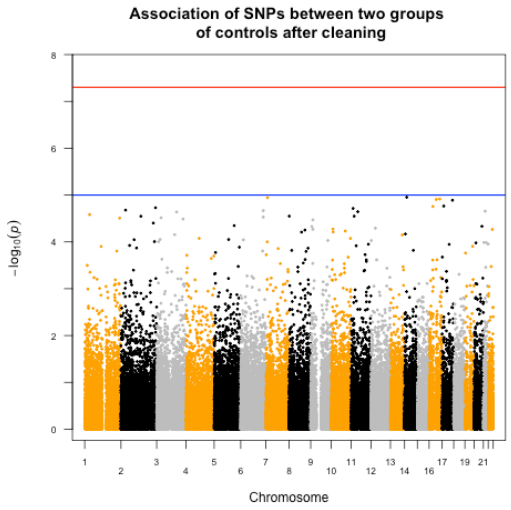
The primary criticism of our approach, using two different chips, would be the concern that between-chip differences, unaccounted for by covariates for ancestry, would lead to

spurious results. We tested this by investigating the genomic inflation, λ, after using 20 dimensions accounting for population structure (calculated using MDS) to covary for effects of ancestry. We calculated dimensions in PLINK by first producing a set of genome-wide SNPs in linkage equilibrium. We removed the MHC (an area of high linkage disequilibrium) and pruned using a sliding window of 50bp, moving by 5bp, and removing SNPs which produce a variance inflation factor (VIF) greater than 2 within that window. This left 47,951 SNPs, which were used to calculate dimensions.

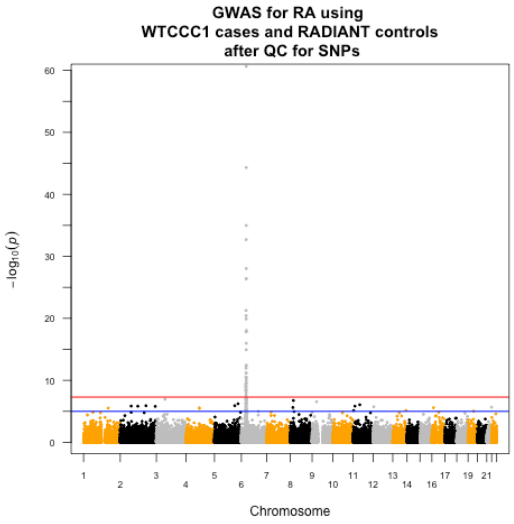
We calculated 20 dimensions, and then regressed control group membership (RADIANT vs WTCCC1) on genotype plus increasing number of dimensions, noting genomic inflation for each model. The genomic inflation factor, a measure of population structure, calculated using the median chi-squared statistic, was $\lambda = 1.0395$ when using 5 dimensions as covariates, indicating that there was minimal population structure which couldn't be accounted for by the use of dimensions accounting for population structure. This is a critical justification for our rationale, as we rely on our ability to assume that differences between cases and controls in our test dataset are due to alleles differentially associated with RA, rather than simply physical differences genotyping chips used.

This case-control analysis is also used to determine the number of eigenvectors necessary to account for population stratification between our case and control datasets. We used multi-dimensional scaling (MDS) to calculate eigenvectors for our merged dataset. MDS requires SNPs in linkage equilibrium. We therefore removed the MHC (26 – 33 Mb on chromosome 6, an area of high linkage disequilibrium), and pruned the remaining SNPs using PLINK-1.07 under the protocol outlined above - using a sliding window of 50bp, moving by 5bp, and removing SNPs which produce a variance inflation factor (VIF) greater than 2 within that window.

S1.a



b



c

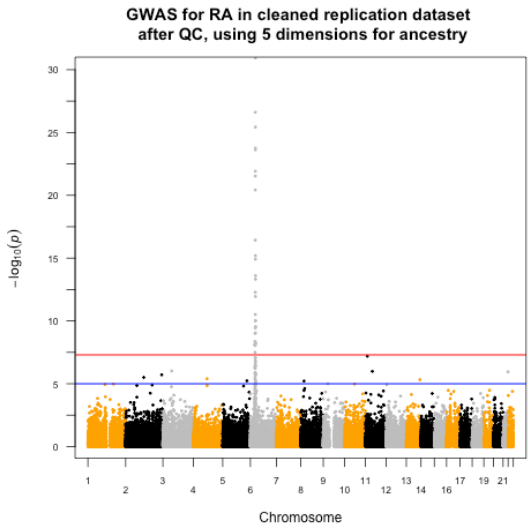


Fig S1:
a. Genome-wide association between the controls used in the test dataset (from the RADIANT study) and the WTCCC controls. We were interested in removing SNPs differing substantially between the platforms these two groups were genotyped on. After cleaning, there was no substantial difference between the two groups

b. GWAS of RADIANT controls vs WTCCC-RA cases. We have replicated the WTCCC's original (2007) result, with a substantially associated region in the MHC on chromosome 6

c. Manhattan Plot of association with RA for with SNPs in cleaned test dataset. *P*-values from logistic regression after using 5 dimensions to account for population structure

We calculated the first 20 dimensions that mapped identity by state across independent SNPs. The first two dimensions showed substantial deviation from expectation (**fig S2**), and so we removed individuals with a score on dimension 1 less than -0.06, or on dimension 3 less than -0.06. This produced a more conventional plot of all the first 4 dimensions. We therefore had 1,989 cases and 1,588 controls remaining – this left 3,577 individuals in our test dataset.

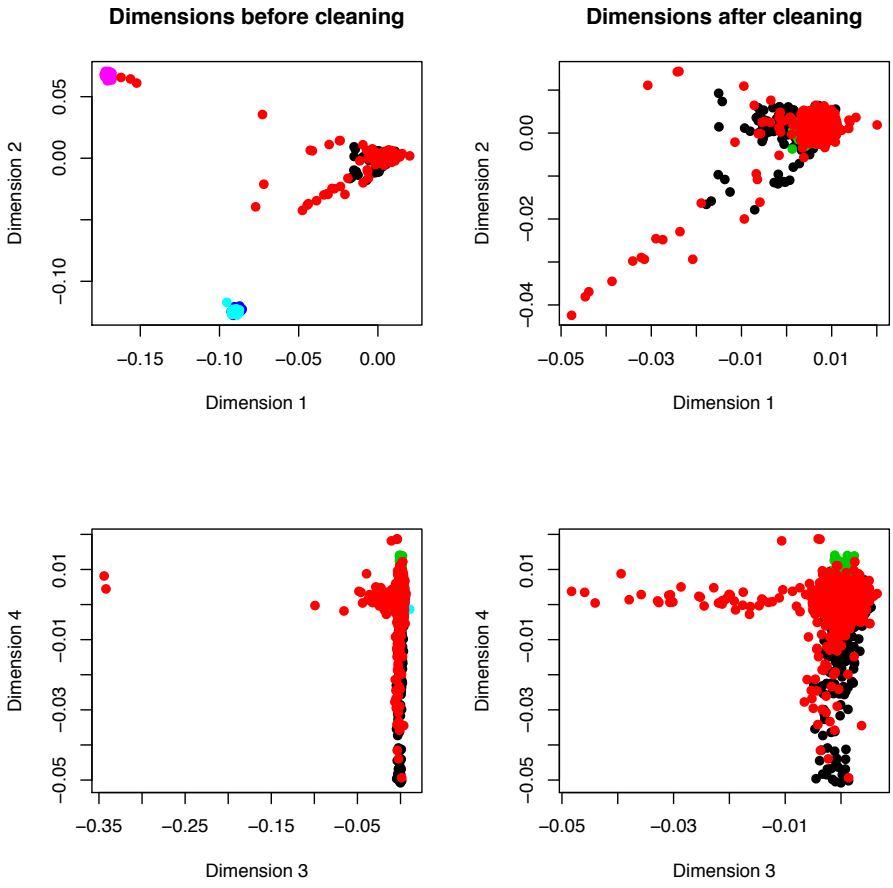


Fig S2: Axes of dimensions accounting for population structure; in all graphs, cases are in red, controls are in black, Europeans (CEU) are in green, Chinese (CHB) are dark blue, Japanese (JPT) are light blue and Yorubans (YRI) are purple. The complete RADIANT controls, Wellcome Trust Cases and HapMap2.3 samples are presented on the left column. We removed individuals with a score on dimension 1 less than -0.06, or on dimension 3 less than -0.06.The remaining individuals are presented on the right column.

This is broadly in line with the Principal Component plots that should be expected under a null assumption of no population stratification. We then ran a series of logistic regression models in PLINK adding increasing numbers of dimensions used as covariates. When using 5 dimensions as covariates, genomic inflation was $\lambda = 1.095$, indicating good control of ancestry or platform differences in the merged data sets. The results of this association test are presented as a Manhattan plot (**fig S1.c)**. The individuals used in the test dataset are summarised below **(table S1)**.

RA Cases				RA Controls				Total
Male	Female	Unknown	Total	Male	Female	Unknown	Total	
498	1491	0	1989	595	993	0	1588	3577

Table S1: Distribution of sex and affection status in test dataset

Supplementary 2: Polygenic Risk Scoring excluding MHC SNP

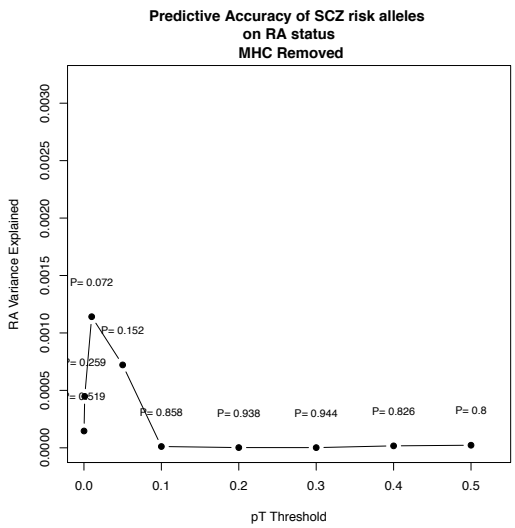
We repeated genome-wide polygenic scoring without the addition of any MHC-SNP. The results of this are below (**table S2, fig S3.a).**

Threshold, P_T	Number of SNPs included	Variance in RA status explained, Pseudo R^2	*P*-Value
0.0001	81	0.0001	0.519
0.001	298	0.0004	0.259
0.01	1,392	0.0011	0.072
0.05	4,450	0.0007	0.152
0.1	7,395	0.0000	0.858
0.2	12,430	0.0000	0.938
0.3	16,707	0.0000	0.944
0.4	20,633	0.0000	0.826
0.5	24,121	0.0000	0.800

Table S2: Proportion of variance in RA status explained by polygenic risk score for SCZ calculated at different thresholds, P_T, using SNPs genome-wide excluding the MHC-region

The upper 5% tails of the distribution, which capture those with high SCZ PRS scores, had similar proportions of RA cases and controls (cases: 5.7%; 95% CI 4.6% - 6.7% compared to controls 5.0%, 95% CI 4.0% - 6.1%) and similar results were seen in the lower 5% tail (**Figure S3.b**). These results suggest that genetic factors do not predict any epidemiological patterns of comorbidity between RA and SCZ.

S3.a | **b**



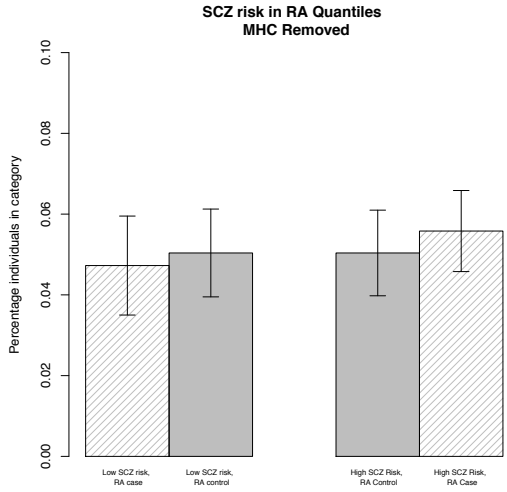


Fig S3.
a. Variance in RA status explained by polygenic risk scores for SCZ calculated using different cut-off thresholds.

b. Proportion of RA cases (lined) and RA controls (grey) in lowest SCZ risk quantile (standardized PRS < -1.66) and in highest SCZ risk quantile (standardized PRS > 1.58).

We explored the logistic model regressing RA status on 5 dimensions accounting for population structure and standardised polygenic risk score ($P_T < 0.01$) for schizophrenia. In this model, the beta for PRS was 0.06. That is to say, a two standard deviation increase in polygenic risk for SCZ has an odds ratio of 1.03 (95% CI: 0.997 – 1.068) for RA risk.

Supplementary 3: Systematic Review and Meta-analysis

We performed a single meta-analysis of all studies extracted above. In addition to analysing the full systematic review results, we subdivided studies by control population. To account for effects of long term institutionalisation and aid in sample collection, many authors used non-schizophrenic psychiatric patients as their control group – a meta-analysis of the incidence of RA between SCZ patients and these reference patients *only* is shown below **(fig S4)**. The effect of SCZ on reducing RA incidence is preserved, indicating a main effect of SCZ, rather that psychiatric illness in general, driving this association.

The negative association between SCZ status and RA prevalence remained statistically significant, random effects OR = 3.43 (95% CI: 0.163 – 0.720, $P = 0.0047$). There was significant heterogeneity between studies ($P = 0.0019$).

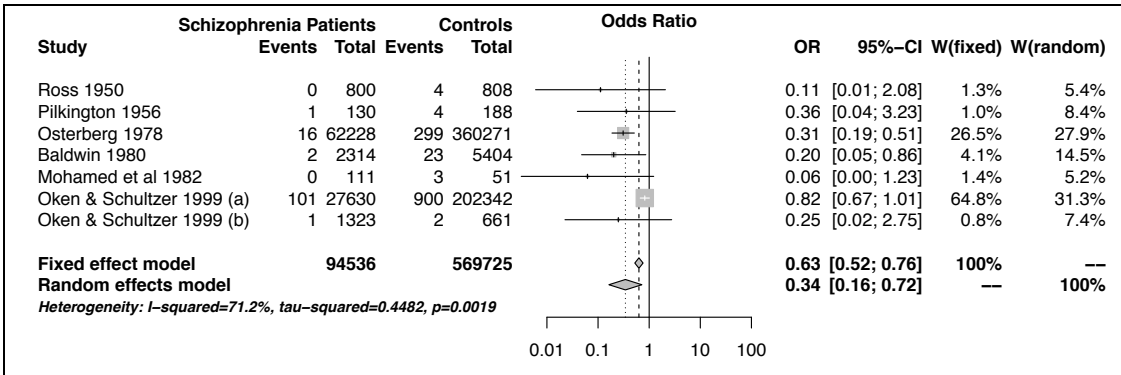


Fig S4: RA prevalence (events) in SCZ cases and non-SCZ psychiatric patients. The original significant protective effect of SCZ on RA reported above was replicated here

Supplementary 4: Using PGC1 SCZ only as Discovery Dataset

4.1 Cleaning Discovery Dataset

In order to use the PGC schizophrenia GWAS results **(table S3)** as a discovery dataset, a few quality control procedures are necessary. We removed SNPs from the GWA results with an info score less than 0.7, indicating poor imputation quality. We then removed SNPs not present in the cleaned test dataset. Finally, in order to obtain SNPs in approximate linkage equilibrium, we used *P*-value and LD-informed clumping, extracting SNPs based on LD in HapMap2 CEU samples. Specifically, we selected the SNP with the lowest *P*-value in each LD block of $r^2 < 0.1$, length ≤250kb, leaving 23,150 independent SNPs in our discovery data sets.

SCZ Cases				SCZ Controls				Total
Male	Female	Unknown	Total	Male	Female	Unknown	Total	
4,731	3,106	22	8,442	10,449	10,933	15	21,397	29,833

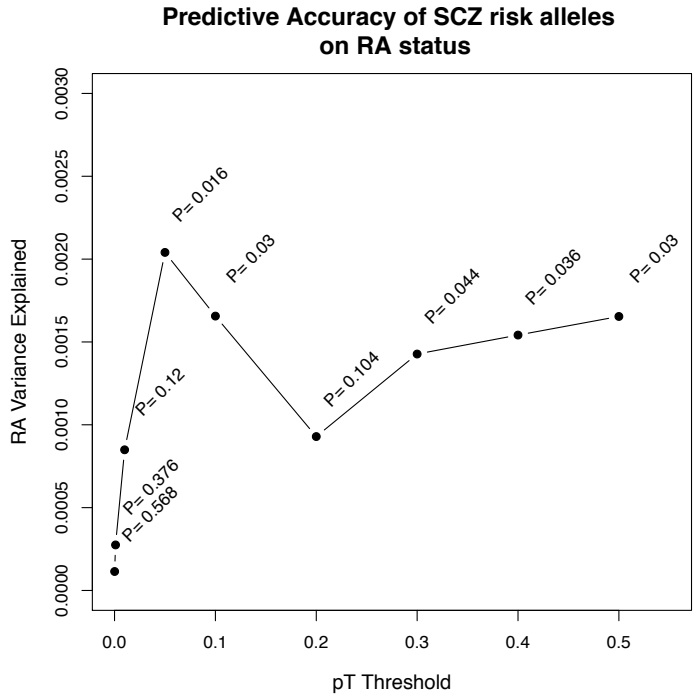
Table S3: Sample characteristics of PGC1 SCZ Study, used as discovery sample for PRS in this section

4.2 Polygenic Scoring

After all quality control, our final test dataset contained genotype data on 1989 cases and 1588 controls. After removing the MHC region (26-33mb on chromosome 6), 23,301 SNPs remained. We calculated the proportion of variance in RA status explained by these polygenic risk scores after removal of the MHC (**table S4, fig S5a**)

We standardised polygenic risk score ($P_T < 0.05$) for schizophrenia – the most predictive threshold (**fig S5.b).** Standardised polygenic scores for SCZ risk were significantly higher in RA cases than controls ($P = 0.0127$).

S5.a



b

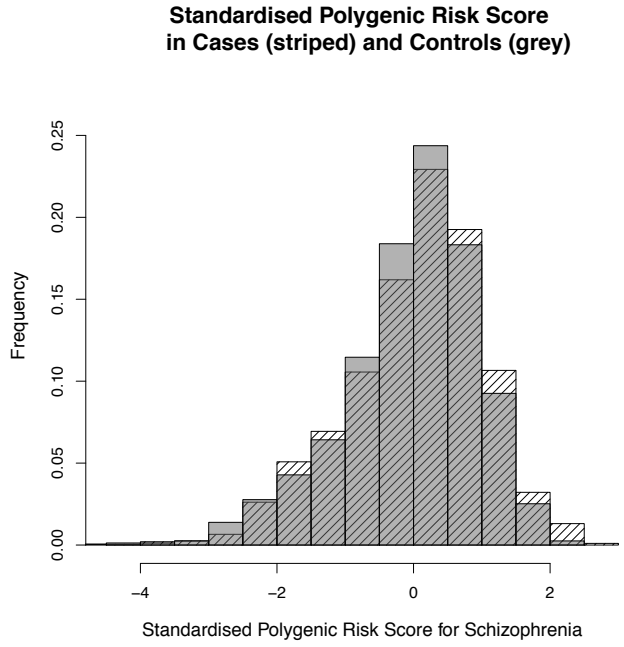


Figure S5:
a. Variance in RA status explained by polygenic risk scores for SCZ calculated using different cut-off thresholds.

b. Distribution of standardized polygenic risk scores in cases (light grey) and controls (dark grey).

Threshold *P*-value, P_T	Number of SNPs	Additional variance explained	*P*-value of Polygenic Risk Score
0.001	276	0.000275	0.568
0.01	1,274	0.000849	0.376
0.05	4,059	0.00204	0.120
0.1	6,880	0.00166	0.0160
0.2	11,778	0.000929	0.0300
0.3	15,955	0.00143	0.0440
0.4	19,812	0.00154	0.0363
0.5	23,301	0.00165	0.0302

Table S4: Polygenic Scoring testing variance in RA status explained by SCZ polygenic risk scores, using PGC1 SCZ data only as a discovery dataset

Supplementary 5: PGC1 + Swedish Data – Sample Characteristics

The PGC1+Swedish dataset contains summary GWAS results for a meta-analysis of two GWAS studies of schizophrenia. The first is the PGC1 SCZ results reported above (table S3) excluding the samples from Sweden – 8,832 cases, 12,067 controls. Secondly the authors performed GWAS of 5,001 cases and 6,243 controls. The results of this analysis are publically available via the PGC. https://pgc.unc.edu/Sharing.php and are analysed here as available on Feb 9th 2014.

Swedish Sample Characteristics	**Cases**	**Controls**
Proportion Male	0.595	0.512
Median Age	54 (45 – 62)	57 (48-65)

Table S5: Swedish sample characteristics. Meta-analysed alongside PGC1 results in order to produce Swedish+ PGC1 dataset.

Supplementary 6: Power Calculations for PRS

We used the polygenescore software developed by Dudbridge to calculate power in our polygene scoring analysis of SCZ risk in RA cases (Dudbridge, 2013). We calculated power to detect shared risk alleles between RA and SCZ at our most predictive score threshold ($P_T < 0.01$) in table S6b; this calculation takes the following parameters (table S6a). We repeat power calculations at an unselected cut-off threshold of $P_T<0.5$ in table S6c in order to investigate the effect of selection bias at the most predictive threshold generating spurious results.

We tested three values of potential correlations between genetic effect sizes, based on genetic pleiotropy work by Lee et al (Lee, Yang, Goddard, Visscher, & Wray, 2012). We calculated power at $\alpha = 0.05$ for genetic correlations of 0.05, 0.1 and 0.15. We also test 5 possible values for proportion of null-SNPs genome-wide, from 0 – indicating all SNPs are causal – to 0.99, as the effect of 100% non-causal SNPs cannot be calculated. We thus obtain 15 estimates for the power of our analysis.

Parameter	Value
Number of samples in discovery dataset	13,833 cases, 18,310 controls
Number of SNPs in analysis	69,621
Number of samples in test dataset	1,989 cases, 1,588 controls
Variance in Discovery dataset explained by all genetic effects (GREML estimate from PGC 2013)	0.26
Variance in Test dataset explained by all genetic effects (GREML estimate from Stahl et al 2012)	0.32
Prevalence of SCZ	0.01
Prevalence of RA	0.006
Proportion Cases in Discovery	0.422
Proportion Cases in Test	0.556

Table S6a: Parameters used for polygenic risk scoring power calculations

Polygenic scoring to investigate genetic overlap between RA and SCZ has power ranging from 0.07 to 1.00 depending on the genetic architecture of the overlap between these two phenotypes, at $\alpha = 0.05$. This indicates our study is well powered to detect pleiotropic effects of the same magnitude observed in other pairs of disorders – for example Dudbridge estimates a genetic effect correlation between SCZ and bipolar disorder of 0.706 (95% CI: 0.513-0.897), (Dudbridge, 2013), assuming a high proportion of SNPs are non-causal.

		Proportion of null SNPs, i.e. those with no genetic effects on phenotypic correlation				
		0	**0.05**	**0.5**	**0.95**	**0.99**
Base-Target genetic effect correlation	**0.05**	0.066	0.067	0.081	0.345	0.495
	0.1	0.117	0.120	0.177	0.878	0.974
	0.15	0.204	0.211	0.338	0.997	1.000

Table S6b: Power for PRS to detect effects in our main analysis, calculated using polygenescore, assuming a number of different values for the correlation between genetic effects in discovery dataset (SCZ GWAS) and test dataset (RA GWAS), and a number of different values for proportion of null SNPs genome-wide

		Proportion of null SNPs, i.e. those with no genetic effects on phenotypic correlation				
		0	**0.05**	**0.5**	**0.95**	**0.99**
Base-Target genetic effect correlation	**0.05**	0.164	0.165	0.167	0.177	0.179
	0.1	0.498	0.499	0.507	0.539	0.544
	0.15	0.835	0.836	0.844	0.870	0.874

Table S6c: Repeating the above power calculations looking at $P_T<0.5$ in order to validate concerns of selection bias on power calculations for best threshold

Finally, we used software provided by Dudbridge to demonstrate that, assuming 5% of SNPs have effects, we have 80% power here to detect a genetic effect correlation of 0.090. Assuming 1% of SNPs have effects, we have 80% power to detect a genetic effect correlation of 0.072. These are much less than genetic effect correlations estimated for canonically pleiotropic conditions with similar epidemiological relationships – such as the estimated genetic effect correlation between SCZ and bipolar disorder of 0.706. Therefore, provided the proportion of null SNPs is high, we would have reasonable power to reject the null in the presence of a genetic effect in this analysis.

Supplementary 7: Direction of effect for SNPs within the MHC

A study using FDR-informed SNP ordering recently demonstrated that SCZ-associated SNPs acted in the same direction as those associated with Multiple Sclerosis more frequently than would be expected by chance – evidence of pleiotropy (Andreassen, Thompson, & Dale, 2014). Furthermore the authors demonstrated that this effect was driven by SNPs in the MHC region. We therefore repeated our direction of effect analysis above, focusing specifically on SNPs within the MHC (26 – 33Mb on Chromosome 6).

After correcting for multiple testing, none of the results of sign tests for shared direction of effect were statistically significant (table S7). Therefore these results are consistent with those above, demonstrating a lack of genetic association between RA and SCZ, and nominal evidence for shared risk alleles.

Clumped By	Threshold, $P <$	N SNPs	P, Pearson's χ^2	Proportion SNPs in same direction
RA	0.01	95	0.201	0.526
	0.1	132	0.025	0.515
	0.2	145	0.090	0.531
	0.3	149	0.129	0.523
	0.4	152	0.152	0.520
	0.5	152	0.152	0.520
SCZ	0.01	83	0.826	0.482
	0.1	114	0.835	0.491
	0.2	128	1.00	0.508
	0.3	136	1.00	0.507
	0.4	142	0.977	0.514
	0.5	148	1.00	0.507

Table S7: Shared direction of effect between independent SCZ and RA risk alleles in the MHC region, across published GWAS

Supplementary 8: Genetic Profile Risk Scoring

SCZ genetic risk scores calculated using the panel of 542 SNPs proposed by Ayalew et al did not associate with RA status (Ayalew et al., 2012). We explored this further by investigating these SNPs within the most recent publically available RA GWAS (Stahl et al., 2010). Within an RA GWAS, the *P*-values for association with RA status were uniformly distributed (Kolmogorov-Smirnoff test *P*-value = 0.13). The SNP panel provided by Ayalew et al orders SNPs based on the genes they lie within. We therefore obtained this list of genes (n=42), and calculated gene-based *P*-values for rheumatoid arthritis using the summary data available from Stahl et al and the utility VEGAS (Liu et al., 2010). RA gene-based *P*-values were uniformly distributed across the 42 SCZ risk genes identified by Ayalew et al (Kolmogorov-Smirnoff test *P*-value = 0.604), indicating that genetic variation across these SCZ risk genes is not associated with RA status.

Displayed on a Manhattan plot of our RA case-control data after imputation and QC, no GPRS SNPs or their proxies show a suggestively significant association ($\alpha = 5x10^{-5}$) with RA (**supplementary figure 6**).

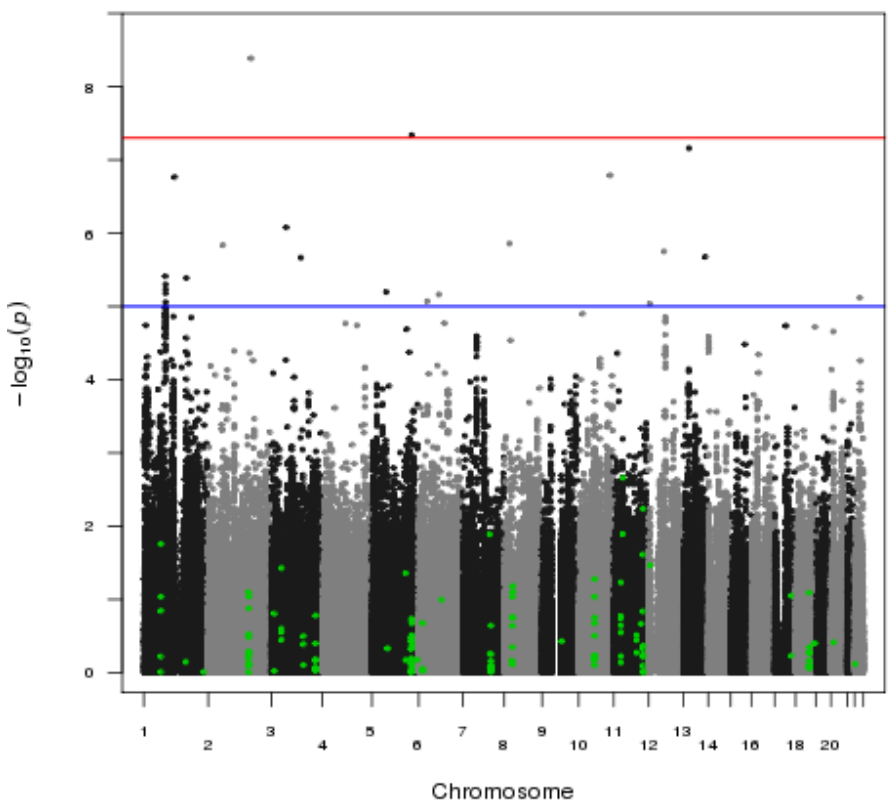


Figure S6: Manhattan plot of imputed SNPs in RA case-control analysis. GPRS542 SNPs and their proxies are highlighted in green.

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Appendix – The Genetic Overlap between PGC2-SCZ and Rheumatoid Arthritis

Introduction

Following the publication of our investigation into the genetic overlap between schizophrenia (SCZ) and rheumatoid arthritis (RA) (Euesden, Breen, Farmer, McGuffin, & Lewis, 2015), there have been two notable developments that allow our study to be updated with higher power to reinforce our conclusions. Firstly, in our original study we constructed Polygenic Risk Scores for Schizophrenia using the PGC1+Swedish Genome Wide Association Study (Ripke et al., 2013). This GWAS is now superseded as the largest publically available Genome-Wide Association Study of Schizophrenia to date by the PGC2 study (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), whose sample characteristics are described below (Table A1). Secondly, we have demonstrated elsewhere (Euesden, Lewis, & O'Reilly, 2015) that the standard Polygenic Risk Scoring (PRS) approach used extensively by others, and in our initial investigation of SCZ and RA, may be improved upon by the use of a larger number of thresholds P_T and any multiple testing burden offset through the use of a more stringent significance threshold of $\alpha = 0.001$. Here we therefore present updated results from our study of SCZ and RA, using the PGC2 SCZ GWAS (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014) for its higher power, and using PRSice with high-resolution scoring for its optimised detection of the most significant threshold for the construction of PRS.

Methods

We used the same RA case-control sample from our original investigation (Euesden, Breen, et al., 2015) as a target data set. Our base sample is the PGC2 schizophrenia data, whose sample characteristics are below (Table A1).

We implement high-resolution scoring in PRSice, at 10,000 thresholds, from P_T=0.001 to P_T=0.5 at increments of P_T=0.0005. All scores are calculated using default clumping protocols in PRSice, with the Major Histocompatibility Complex removed. Scores are regressed on rheumatoid arthritis status at each threshold; these logistic models are adjusted for the first 5 MDS dimensions as calculated and reported previously (Euesden et al 2015).

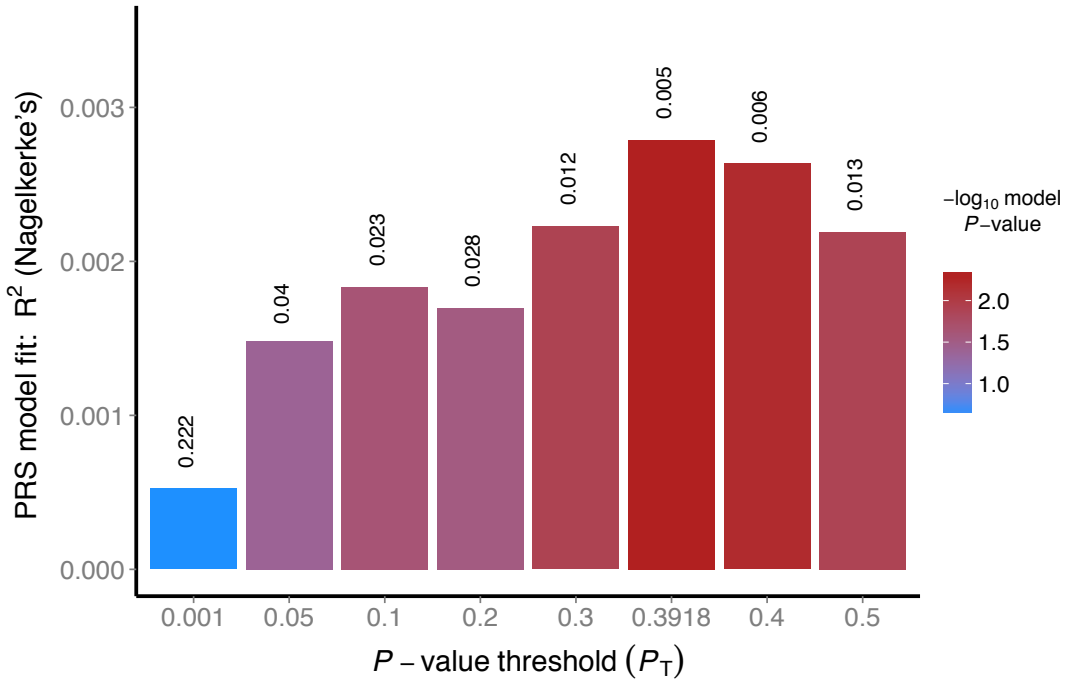
Dataset	**SCZ Cases**			**Controls**			**Individuals in Trios**	**Total**	**#GWAS Hits**
	European	Asian	Total	European	Asian	Total			
PGC2	32,405	1,836	34,241	42,221	3,383	45,604	2,470	82,315	128
PGC1+ Swedish	13,833		13,833	18,310		18,310		32,143	22
PGC1	9,394		9,394	12,462		12,462		21,856	7

Table A1: Sample characteristics of the PGC2 schizophrenia data used as base in this supplementary analysis. As comparison, the PGC1 sample, presented in the supplementary materials to chapter 3 is included. These individuals are meta-analysed with a sample of 8,832 SCZ cases and 12,067 controls recruited from Sweden to give the final analytic sample of 13,833 SCZ cases cases and 18,310 controls that are used in the main analysis in chapter 3. The number of Genome-Wide significant loci identified in each study is also presented for illustration of the relative power of these studies

Results

At the most predictive threshold, P_T=0.3918, Schizophrenia PRS predicts rheumatoid arthritis case status with a *P*-value of 0.005. Whilst suggestively significant, this lies above our suggested α threshold of 0.001, which is necessary to adequately account for the multiple testing burden resulting from the use of 10,000 *P*-value thresholds for PRS calculation. Furthermore, as in our previous analysis, we find a nominally positive effect of SCZ PRS predicting RA case status. For a 1-standard deviation increase in SCZ PRS, the odds ratio for RA is 1.11 (99.9% Confidence Interval – corresponding to α = 0.001 – is 0.98 – 1.24), suggesting that it is unlikely that increased SCZ PRS is reducing risk of rheumatoid arthritis in the general population, or that this could contribute to the epidemiological relationship we have reported, which is in the opposite direction. These results are displayed below graphically (figure A1) and at a selected number of thresholds (table A2).

a)



b)

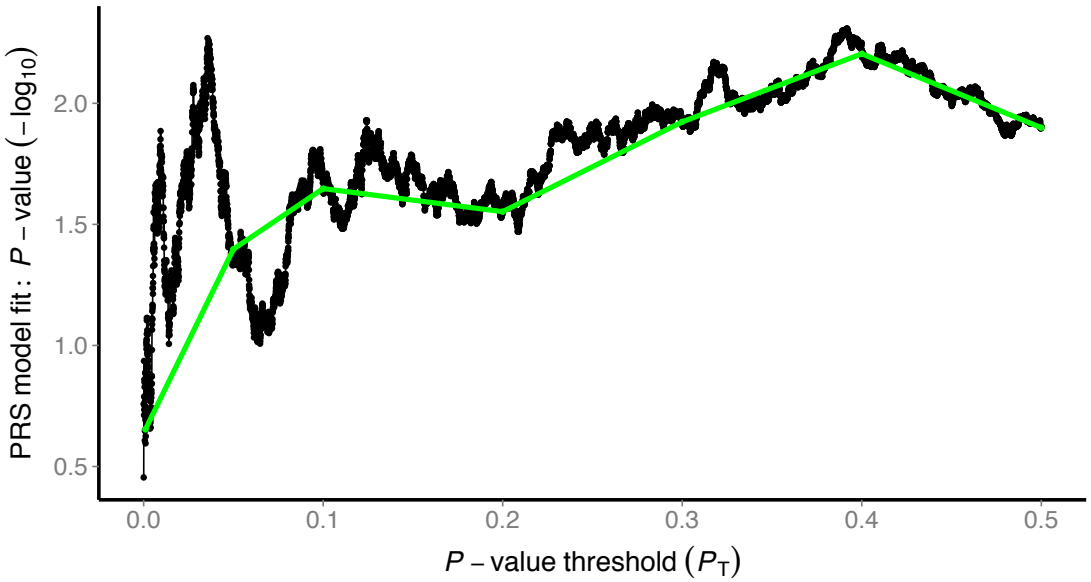


Figure A1: PRS for Schizophrenia predicting rheumatoid arthritis case-control status across thresholds. Results using high-resolution scoring are presented (a) as a bar chart and (b) as a point-plot with the results of PRS at a limited number of thresholds superimposed in green to demonstrate the increased resolution allowed by PRSice .

	PGC2 Data			PGC1+Swedish Data		
P_T	***P*-value**	**Variance explained**	**Number of SNPs**	***P*-value**	**Variance explained**	**Number of SNPs**
0.001	0.2220	0.0005	771	0.276	0.0004	299
0.05	0.0400	0.0015	5776	0.154	0.0007	4,451
0.1	0.0225	0.0018	8695	0.799	0.0000	7,396
0.2	0.0280	0.0017	13477	0.816	0.0000	12,431
0.3	0.0119	0.0022	17316	0.863	0.0000	16,708
0.3918	0.0049	0.0028	20446	NA	NA	NA
0.4	0.0062	0.0026	20690	0.770	0.0000	20,634
0.5	0.0126	0.0022	23652	0.751	0.0000	24,122

Table A2: Results of SCZ PRS predicting RA case-control status at a select number of *P*-value cut-off thresholds including the most significant threshold of P_T=0.3918. We present threshold, P_T used to select SNPs for PRS construction, the *P*-value for SCZ PRS predicting RA Case-Control Status ($\alpha = 0.001$), The variance in RA Case-Control Status explained by SCZ PRS (Nagelkerke's Pseudo R^2), and the number of SNPs (N) included in scores calculated at this threshold. No thresholds produce scores that are significant predictors of RA status at our suggested α threshold of 0.001. For comparison, results are presented alongside those obtained from the PGC1+Swedish data in chapter 3 – NB we do not present the results at P_T=0.3918 in the PGC1+Swedish data as this is derived from high-resolution scoring.

Discussion

We find, in line with our previous analyses, that there is no evidence for a significant genetic overlap between schizophrenia and rheumatoid arthritis. This supports our previous assertion that the relationship observed in epidemiological cohorts may be driven by non-genetic factors, such as a harvesting effect due to the decreased life expectancy of schizophrenia patients, or an effect of medication whereby antipsychotics are reducing an individual's risk of subsequently presenting with rheumatoid arthritis.

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Chapter 4: A bidirectional relationship between depression and the autoimmune disorders - new perspectives from the National Child Development Study

A bidirectional relationship between depression and the autoimmune disorders – new perspectives from the National Child Development Study

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Word Count: 3,803

Abstract

Background

Depression and the autoimmune disorders are comorbid - the two classes of disorders overlap in the same individuals at a higher frequency than chance. The immune system may influence the pathological processes underlying depression; understanding the origins of this comorbidity may contribute to dissecting the mechanisms underlying these disorders.

Method

We used population cohort data from the National Child Development Study to investigate the ages at onset of depression and 23 autoimmune disorders. We used self-report data to ascertain life-time history of depression, autoimmune disorders and their ages at onset. We modelled the effect of depression onset on subsequent autoimmune disorder onset, and vice versa, and incorporated polygenic risk scores for depression and autoimmune disorder risk.

Results

In our sample of 8174 individuals, 315 reported ever being diagnosed with an autoimmune disorder (3.9%), 1499 reported ever experiencing depression (18.3%). There was significant comorbidity between depression and the autoimmune disorders (OR = 1.66, 95% CI = 1.27-2.15). Autoimmune disorder onset associated with increased subsequent hazard of depression onset (HR = 1.39, 95% CI = 1.11 – 1.74, $P = 0.0037$), independently of depression genetic risk. Finally, depression increased subsequent hazard

of autoimmune disorder onset (HR = 1.40, 95% CI = 1.09 – 1.80, $P = 0.0095$), independently of autoimmune disorder genetic risk.

Discussion

Our results show a bidirectional relationship between depression and the autoimmune disorders. This suggests that shared risk factors may contribute to this relationship, including both common environmental exposures that increase baseline inflammation levels, and shared genetic factors.

Introduction

An epidemiological link between psychiatric and autoimmune disorders has been observed for almost a century (Nissen & Spencer, 1936). The mechanism underlying this overlap is unclear, particularly in depression, one of the most common psychiatric disorders (Kessler et al., 2005).

Several relatively small-scale clinical studies have explored the association between depression and specific autoimmune disorders. In rheumatoid arthritis, multiple sclerosis and the inflammatory bowel diseases, authors have robustly demonstrated an increased overlap between depression and autoimmune disorder diagnosis in the same individuals, above that expected from their prevalences (Dickens, McGowan, Clark-Carter, & Creed, 2002; Kurina, Goldacre, Yeates, & Gill, 2001; Patten, Beck, Williams, Barbui, & Metz, 2003). These relatively small clinical studies have been supplemented by a recent Danish population-based study, which reported that depression is associated with a significantly increased risk of subsequent autoimmune disease (IRR= 1.25, 95% CI 1.19–1.31) (Andersson et al., 2015).

In addition to any clinical implications, investigating the relationship between depression and autoimmune disorders, and identifying the factors driving it, will inform theories that the aetiology of depression involves immune processes (Raison, Capuron, & Miller, 2006; Raison & Miller, 2013). In rheumatoid arthritis, low mood may predict subsequent worsening of symptoms in autoimmune disorder patients (Euesden et al, submitted). The aetiology and pathophysiology of depression is currently poorly understood, and current pharmacological treatments lack efficacy for mild to moderate depression (Fournier et

al., 2010). It is therefore of great clinical importance to identify the mechanisms responsible for the onset of depression, and leverage this information in future work such as the repositioning of pharmaceuticals.

One approach to dissecting this relationship is to examine the relative ages at onset of the two disorders in order to infer elements of causality. If one disorder consistently precedes another, it may reliably increase risk of the second. This has long formed a criterion for establishing causality between two events, as first proposed by Hume and expanded on by others (Holland, 1986). If there is no clear trend in the order of disorder onset, there are two possible interpretations. Firstly, a shared environmental risk factor may increase risk of both disorders. Secondly, this relationship may be due to pleiotropy, common genetic risk factors increasing risk of both disorders, as is seen between depression and a number of other comorbid psychiatric disorders such as schizophrenia (Cross-Disorder Group of the Psychiatric Genomics, 2013).

As far as we are aware, only one study has explored relative ages at onset in depression and the autoimmune disorders to date. Depression has been shown to elevate hazard of autoimmune disorder onset in a Danish population cohort, using confirmed hospital diagnoses to identify cases (Andersson et al 2015). This study design has the advantage of minimising ascertainment bias and any confounding effects of attrition. However, relying on date of first clinical contact as an indicator of age at onset may estimate an artificially late age at onset, as there is typically a latency between depression onset and clinical diagnosis (Beiser, Erickson, Fleming, & Iacono, 1993).

In this study, we use data from the National Child Development Study (NCDS), a large epidemiological cohort comprised of all children born in England, Scotland and Wales,

in one week of 1958. This sample has been followed up through their adult lives, providing data that allow the temporal analysis of onset of depression and autoimmune disorders. In addition, the availability of genetic data on NCDS cohort members enabled us to examine the contribution of genetic risk to disease onset, alongside traditional epidemiological methods. Risk of both depression and almost all autoimmune disorders studied to date including type 1 diabetes, rheumatoid arthritis,ankylosing spondylitis, Crohn's disease, psoriasis, primary sclerosing cholangitis and ulcerative colitis are influenced by large numbers of common polymorphisms (Single Nucleotide Polymorphisms, SNPs) of small effect, which often act to increase risk of a number of phenotypes concomitantly (Bulik-Sullivan et al., 2015; Ellinghaus et al., 2016; Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013; Okada et al., 2014; Wellcome Trust Case Control Consortium, 2007). We therefore investigated the role of these common genetic risk factors within a longitudinal population cohort in order to dissect environmental and genetic risk factors influencing the relationship between depression and autoimmune disorders.

Materials and Methods

Sample

We used data from the National Child Development Study (Power & Elliott, 2006), a sample of the 17,638 individuals born in Scotland, England and Wales in one week of 1958. This cohort has been followed up on multiple occasions across childhood and in adulthood. We used self-report data from waves 5 (age 33), 6 (age 42) and 7 (age 46), collected in the years 1991, 2000 and 2004, along with genotype data derived from the biomedical survey undertaken in 2002-4, when cohort members were aged 44-45 years. Ethical approval was given by the South East Multi-Centre Research Ethics Committee.

Measures

Autoimmune Disorders

In wave 7 of the NCDS, participants were queried about their medical histories via telephone interview; disorders were stored as ICD-10 codes alongside self-reported ages at onset. We investigated the following 23 autoimmune disorders, pooling them to form a single autoimmune disease phenotype: Addison's disease, autoimmune haemolytic anaemia, autoimmune thrombocytopenia purpura, celiac disease, dermatomyositis, Graves' disease, Hashimoto's thyroiditis, idiopathic myocarditis, idiopathic pulmonary fibrosis, insulin-dependent diabetes mellitus, inflammatory bowel disease (Crohn's disease and ulcerative colitis), multiple sclerosis, myasthenia gravis, pernicious anaemia, polyarthritis, psoriasis, rheumatoid arthritis, scleroderma, Sjogren disease, systemic lupus erythematosus, vitiligo, and Wegener's granulomatosis. We pool disorders in order to increase power due to many autoimmune disorders being rare individually, and to mitigate any biases introduced through possible misclassification within the autoimmune

disorders during interview. Participants were considered *unexposed* before autoimmune disease onset (or first autoimmune disease if a participant reported more than one), and *exposed* at age at onset and thereafter. All data were considered censored at age 46, the time-point of the most recent biomedical investigation.

Depression

We drew on three measures of depression onset in the main analysis. In wave 5 (age 33), participants were asked if depression had ever been a problem, and *if so* at what age it was first a problem. In wave 6 (age 42), participants were asked the age they had started feeling depressed. Finally, in wave 7 (age 46), psychiatric histories were taken alongside age at onset (Table 1).

Participants were considered exposed from their earliest report of depression and exposed thereafter; as for the autoimmune disorders, reports of depression onset were censored at age 46. We took a number of steps to ensure the consistency in reports of age at onset across sweeps, and excluded any cases with inconsistent reports (see Supplementary 3.

Genotyping

Blood samples and consent for genotyping were collected in the course of the biomedical survey. Genome-wide genotype data from a subset of NCDS participants were available from previous studies using five different genotyping chips. Quality control was performed on each chip separately: SNPs were removed based on MAF < 1%, Hardy Weinberg equilibrium $P < 10^{-5}$, and missingness > 1%. Individuals with missingness > 10% were removed. In our phenotype-cleaned data set, 5762 participants

were genotyped in total: 2896 participants were genotyped on the Illumina Immunochip, 1271 on the Illumina 1.2M chip, 1456 on the Infinium Humanhap, and 139 on the Affymetrix v6 chip.

Polygenic Risk Scores - Depression

Polygenic Risk Scores (PRS) for depression for each genotyped NCDS participant were calculated using genome-wide results from the Psychiatric Genomics Consortium MDD study (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013). PRS give a measure of genetic risk for each individual by summing the number of risk alleles carried, weighted by the natural logarithm of the odds ratio for each SNP as identified in GWAS. PRS were calculated using PRSice, including SNPs reaching *P*-value threshold of $P_T = 0.3$, previously shown to produce a reliable predictor of MDD in independent samples (Major Depressive Disorder Working Group of the Psychiatric GWAS Consortium et al., 2013; Peyrot et al., 2014); (Euesden, Lewis, & O'Reilly, 2015). PRS were computed by genotyping chip and standardised to enable data to be pooled.

Polygenic Risk Scores – Rheumatoid Arthritis

Of publically available GWAS results for autoimmune disorders, rheumatoid arthritis (Okada et al., 2014) is one of the highest powered by both sample size - 29,880 RA cases and 73,758 controls - and the number of genome-wide significant variants identified (n=101). The NCDS contributed 1,999 controls to the WTCCC study (Wellcome Trust Case Control Consortium, 2007) which is part of the RA GWAS meta-analysis; we

therefore removed the effect of these samples from the Okada GWAS summary statistics by performing an association study on the WTCCC rheumatoid arthritis cases and controls, and perform an inverse meta-analysis in order to obtain a GWAS summary statistics which can be used to calculate PRS in the NCDS – this cleaning of GWAS data is outlined in detail in supplementary section 4.

The summary results from this adjusted RA GWAS meta-analysis were used to construct rheumatoid arthritis PRS for all genotyped NCDS individuals. To obtain the optimum SNP *P*-value threshold, we identified all NCDS rheumatoid arthritis and polyarthritis cases, and classified others as controls. We tested the ability of rheumatoid arthritis PRS to predict this case-control arthritis status at a number of SNP *P*-value thresholds. Logistic regression for case-control status was performed for each chip separately, and the PRS regression coefficient was meta-analysed across chips at each *P*-value threshold. This showed that the most predictive threshold was P_T=0.001, consistent with the high power of the RA GWAS meta-analysis (Dudbridge, 2013). RA PRS at this *P*-value threshold were standardised by chip and used in modelling below.

Statistical Analyses

All analysis, unless stated otherwise, was performed using R version 3.2.2 (R Development Core Team, 2008). We fitted Cox Proportional Hazards models to investigate the time-course of depression on autoimmune disorder onset and vice-versa, using Breslow's method to estimate the baseline hazard function. In each case, the predictor variable is coded as a time-varying covariate; this is achieved by specifying multiple observations per individual, one before any exposure, one before any outcomes and a third thereafter. Individuals were considered unexposed the year before reported

age at onset for a phenotype and exposed thereafter. For example in testing for the effect of depression on age at onset of autoimmune disorder, a participant reporting depression onset at age 20 and an autoimmune disorder at age 40 would be considered unexposed for depression until age 20, exposed for depression but not autoimmune disorder up to age 40, and then exposed for both depression and autoimmune disorder until age 46, the most recent point at which data was collected. Models were fitted using autoimmune disorder as the event, coding by exposure to depression at different time points, and similarly using depression as the event, coding for diagnosis of autoimmune disorder. All models were adjusted for gender as this is a strong predictor of both depression (Weissman & Klerman, 1977) and autoimmune disorder (Whitacre, Reingold, & O'Looney, 1999). For each model, we tested the null hypothesis that the exposure (depression, autoimmune disorder) had no effect on the event (autoimmune disorder, depression). Finally, PRS for MDD were incorporated into the Cox Proportional Hazards models in order to estimate the genetic contribution to hazard of depression – firstly in a univariate model investigating hazard of depression and secondly in a multivariate model adjusting for any effect of autoimmune disorder onset; we then incorporate the same procedure to test the effect of PRS for rheumatoid arthritis predicting hazard of autoimmune disorder onset, extending this to a multivariate case where the onset of depression is adjusted for.

Results

Overlap between autoimmune disorders and depression

After harmonisation across time-points and data cleaning, 8174 individuals (48% female) remained in our analytic sample. By age 46, 315 (3.85%,; 55.6% female) reported ever being diagnosed with an autoimmune disorder (an event-rate per 10,000 person-years of 8.38), and 1499 (18.3%; 65.6% female) were positive for our measure of depression. Depression and autoimmune disorders co-occurred in the same participants at a higher rate than would be expected by chance (84 individuals, Odds Ratio = 1.66, 95% CI = 1.27 – 2.15, Fisher's exact test $P = 1.92 \times 10^{-4}$).

Dissecting Directionality via Relative Ages at Onset

The mean reported age at onset for autoimmune disorders was 33.2 years (SD=10.9), and for depression was 34.4 years (SD=6.3). Reported ages at onset were not significantly different for men and women for autoimmune disorders ($P = 0.105$), and were significantly later in males than females in depression ($P = 6.93 \times 10^{-6}$). The ages at onset of depression and the autoimmune disorders are shown on histograms in figure 1, on the x and y axes respectively; ages at onset in individuals with both disorders are shown as points, with darker points indicating multiple individuals with the same combination of ages at onset.

Cox Proportional Hazards models allow us to explore time-dependent changes in hazard, and to use time-varying covariates. We fitted Cox Proportional Hazards models treating autoimmune disorder as a time-varying covariate for depression onset, and depression

onset as a time-dependant covariate for autoimmune disorder onset. Autoimmune disorder onset increased the hazard of subsequent depression onset, with a Hazard Ratio of 1.39 (95% CI = 1.11 – 1.74, $P = 3.7 \times 10^{-3}$), adjusting for sex. We also found evidence for an effect of depression onset increasing subsequent hazard of autoimmune disorder onset (HR = 1.40, 95% CI = 1.09 - 1.80, $P = 9.5 \times 10^{-3}$), adjusting for sex. These results are displayed graphically as Kaplan-Meier curves (figure 2).

Shared genetic risk

To test for shared risk genes increasing hazard of both depression and the autoimmune disorders, we incorporated PRS from genotype data on subset of the NCDS sample (N = 5762). As individuals were genotyped on one of 5 chips, we verified that standardised Polygenic Risk Scores (PRS) for depression did not differ across chips (ANOVA F = 0.57, *P*–value = 0.69). In a univariate model, MDD PRS was a significant predictor of depression hazard (HR = 1.09, 95% CI = 1.03 – 1.12, $P = 5.3 \times 10^{-3}$), and after adjusting for gender (HR = 1.08, 95% CI = 1.02 – 1.15, $P = 9.1 \times 10^{-3}$).

Including autoimmune disorder onset and MDD PRS in a Cox model adjusting for gender, we found that both MDD PRS (HR = 1.08, 95% CI = 1.02 – 1.15, $P = 8.7 \times 10^{-3}$) and autoimmune disorder onset (HR = 1.32, 95% CI = 1.01 – 1.72, $P = 0.046$) were significant, independent predictors of subsequent depression onset. Although the smaller Ns in the genotyped sample reduced the statistical power of this model, the point estimate for the Hazard Ratio of autoimmune disorder status on depression onset was closely similar to that in the phenotypic analysis reported above.

For RA PRS, we confirmed that scores did not differ by genotype chip (ANOVA F = 0.93, *P*-value=0.45), and that it predicted rheumatoid arthritis and polyarthritis case-control status (*P*=0.005). In a Cox Proportional Hazards model adjusting for gender and depression onset, RA PRS predicted autoimmune disorder hazard (HR = 1.15, 95% CI = 1.01 – 1.31, *P* = 0.03), with a nominally significant independent effect of depression status (HR = 1.31, 95% CI = 0.97 – 1.38, *P* = 0.08).

Whilst the effect of depression onset predicting AD onset is not statistically significant when adjusting for RA PRS, it is likely that this is due to power concerns. Firstly, we demonstrate below that RA PRS does not predict depression status, and so it is unlikely that adjusting for RA PRS accounts for any effect of depression predicting AD onset. Secondly, the model adjusted for RA PRS has reduced power to detect modest effect sizes, a general principle of models with dichotomous outcomes and additional covariates predicting outcome but not exposure (Mefford & Witte, 2012). Thirdly, the hazard ratio for depression predicting subsequent AD onset estimated when adjusting for RA PRS (HR = 1.31) remained similar to the hazard ratio obtained before adjustment (HR=1.40). Thus our interpretation of the effect of depression predicting subsequent AD onset after adjusting for RA PRS as suggestively significant (*P*=0.08) appears broadly justified.

We finally tested whether RA PRS predicted depression onset, and whether MDD PRS predicted AD onset. Neither of these associations were significant: HR = 0.99 (*P*=0.79) and HR = 0.97 (*P*=0.68) respectively. We repeat all of the above analyses using cluster robust standard errors in order to account for the effect of longitudinal dependence across observations. The results of these analyses are presented – in comparison with the

results of the above analyses – in supplementary 5 and table S3, and confirm that accounting for the effects of this dependence has little effect on our results.

Discussion

We explored the relationship between depression and autoimmune disorders up to mid-life using epidemiological and genetic data. We replicated the finding that autoimmune disorders are frequently comorbid with depression, using a longitudinal national birth cohort and self-report data, which is increasingly used in the study of depression (Smith et al., 2013). We also demonstrated an effect of autoimmune disorder onset increasing hazard of subsequent depression onset; this effect was independent of the effect of genetic risk factors influencing hazard of depression. These results highlight the utility of a longitudinal approach to problems of medical comorbidities in epidemiology; our epidemiological results replicate those of Andersson et al (2015), and build on them by including individuals who had not sought specialist mental health care, but who had explicitly answered questionnaires on history and current status of depression, within a population-based sample. There are a number of alternative explanatory models for the observed findings

Causative effect of depression on autoimmune disorders

Longitudinal studies have shown that depression shows a two-way association with systemic inflammation (Matthews et al., 2010), which is a key component in the pathophysiology of autoimmune disorders, such as rheumatoid arthritis (Smolen, Aletaha, Koeller, Weisman, & Emery, 2007). Our finding that depression onset can increase subsequent hazard of autoimmune disorder onset is consistent with this model (Sheehy, Murphy, & Barry, 2006).

Causative effect of autoimmune disorders on depression

The role of immune abnormalities in the pathophysiology of depression has been the focus of intense research for the past two decades (Miller & Raison, 2015). For example, experimental stimulation with pro-inflammatory cytokines and bacterial compounds, such as lipopolysaccharides (LPS), induces a cluster of symptoms overlapping with depressive symptoms in animal models and humans (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Musselman et al., 2001). Furthermore, anti-inflammatory medications appear to have antidepressant effects (Kohler et al., 2014). Our finding that autoimmune disorder onset can increase subsequent hazard of depression onset is consistent with these findings.

Shared Environmental Factors

Many behaviours associated with MDD, such as smoking (Bjørngaard et al., 2013) are also associated with autoimmune disorders such as rheumatoid arthritis (Pedersen et al., 2006). Whilst there is little debate that smoking increases risk of RA (Di Giuseppe, Discacciati, Orsini, & Wolk, 2014), there is considerable debate as to the direction of any causal association between smoking and MDD. Bjorngaard et al found no evidence for a causal effect in MDD but negative results in Mendelian randomisation studies are difficult to interpret. The large number of behaviours associated with MDD and the multifactorial nature of both MDD and many autoimmune disorders make it difficult to differentiate between autoimmune disorder risk factors caused by MDD, and common risk factors shared by both MDD and the autoimmune disorders, however temporal ordering may provide one route. There is robust evidence for an association between childhood maltreatment and both depression and the autoimmune diseases (Dube et al., 2009; Nanni, Uher, & Danese, 2012) and immune abnormalities appear concentrated

within a subgroup of depressed individuals with a history of childhood maltreatment (Danese et al., 2008); (Danese et al., 2011). Therefore, early life stressors may play a role in the comorbidity of these two outcomes.

Shared Genetic Factors

Inflammatory models of MDD would suggest a predisposition to higher inflammatory activity, similar to the inflammatory arthritis that precedes rheumatoid arthritis, in depression patients (Raison et al., 2006). Genetic risk of inflammatory over-activity may underlie the epidemiological relationship between these two families of disorders, however this conclusion would not be consistent with our results, as we found no evidence for genetic risk of rheumatoid arthritis increasing hazard of depression. Instead, we find evidence for independent effects of MDD genetic risk and autoimmune disorder onset on hazard of subsequent depression.

We note that two separate causal mechanisms, one by which depression increases hazard of autoimmune disease and another by which autoimmune disease onset increases hazard of depression is a less parsimonious conclusion than the presence of a shared environmental risk factor.

Clinical Implications

Because depression can exacerbate systemic inflammation (Matthews et al., 2010) and symptoms of autoimmune disorders (Sheehy et al., 2006), assessment and treatment of depression in patients with autoimmune disorders is crucial. In addition, not all patients with depression benefit from currently available treatments (Fournier et al., 2010) (Nanni et al., 2012), and new treatments targeting patient subgroups with identified

vulnerabilities, such as immune abnormalities, could offer innovative, effective strategies (Kohler et al., 2014); (Raison & Miller, 2013). Finally, further investigations of shared pathways that may drive the observed comorbidity, including the roles of childhood maltreatment, can uncover key biological mechanisms (Danese & McEwen, 2012).

Limitations

Our findings need to be viewed in light of several limitations. First, the low prevalence of the individual autoimmune disorders - although consistent with rates expected in community samples - required that we group them together into a single composite phenotype. Whilst it has the advantage of mitigating confounding due to misclassification within the autoimmune disorders and has increased power for modelling, this pooled autoimmune disorder phenotype prevented us detecting aetiologically distinct subgroups within autoimmune disorders. It also precluded our investigating the role of the genetics of autoimmune disorders in depression onset, beyond the marker for genetic risk of rheumatoid arthritis used in the present study.

A further limitation arising from our pooled autoimmune disorder phenotype is the difficulty in comparing reported prevalences by disorder. The event-rate for first autoimmune disorder in our sample is 8.38 per 10,000 person-years. This is similar to the same event-rate (8.8) reported for any autoimmune-disorder hospitalisation by Dube et al (2009) between ages 19 and 44 and suggests that self-report is not leading to an over-reporting of medical history in our sample.

A second limitation of this work is the reliance on self-report for indicators of both disease status and age at onset. Our confidence in the validity of our depression measure

comes from several sources. The prevalence of depression in our sample at 18.3% is consistent with previous reports in high-income countries, for example, Bromet et al (Bromet et al., 2011) report 14.6% (SD = 0.2). The higher prevalence of depression in our sample may indicate that the repeated interviews minimise under-reporting of depression, as previously noted by Moffitt et al (Moffitt et al., 2010). As expected, we see later onset for depression in males, and a higher prevalence in females (Burke, Burke, Regier, & Rae, 1990; Van de Velde, Bracke, & Levecque, 2010). We note that our sample has a later average age at onset (34.4 years) than reported elsewhere (Kessler & Bromet, 2013); if this reflected a systematic bias in our study, it would mask any causative effect of depression on autoimmune disorder onset, as depression onset would be reported artificially late in individuals with both disorders. Therefore, our depression measure appears to be reliable, and any possible systemic bias would not detract from our conclusions.

Summary

We have replicated, and built on, previous studies in finding a significant association between major depressive disorder and the autoimmune disorders in an unselected, population-based sample. Furthermore, we have found significant evidence for depression temporally preceding autoimmune disorders in some patients, and vice versa. This suggests a causal effect of MDD on autoimmune disorder onset, perhaps via some depression-associated behaviour such as diet, or an environmental risk factor shared between the two phenotypes, such as cigarette smoking. Finally, we have used genetic data to demonstrate independent effects of autoimmune disorder status and MDD genetic risk scores on onset of depression.

Tables and Figures

Wave	Age (year)	Depression Measure	Number of reported cases (% female)	Number of new cases (% female)
5	33 (1991)	Age at which depression was first a problem	390 (79.0%)	390 (79.0%)
6	42 (2000)	Age first started feeling depressed	1466 (66.1%)	1085 (61.5%)
7	46 (2004)	ICD codes for medical disorders and self reported age at onset. F32, 33 and 34	101 (62.3%)	24 (37.5%)

Table 1: Summary of depression metrics used to determine depression status and age at onset within the NCDS sample.

Measure	Full Sample	Genotyped Subsample
Sample Size	8174	5762
Female (%)	3919 (47.9%)	2902 (50.4%)
Number depressed (%)	1499 (18.3%)	1067 (18.5%)
Number (%) depression cases Female	984 (65.6%)	736 (69%)
Average depression age at onset (SD)	34.4 (6.3)	34.4 (6.36)
Average depression age at onset in women (SD)	33.9 (6.54)	33.8 (6.62)
Number with an autoimmune disorder (%)	315 (3.85%)	226 (3.92%)
Number (%) autoimmune disorder cases female	175 (55.6%)	131 (58%)
Average autoimmune disorder age at onset (SD)	33.2 (10.9)	33.5 (11)
Average autoimmune disorder age at onset in women (SD)	34.1 (10.4)	34.4 (10)

Table 2: Sample characteristics. Measures are presented separately for the full sample, and the subsample that have been genotyped – these are the participants used in the Polygenic Risk Score analysis. All controls are censored at age 46, so case status status denotes onset before this age, and age at onset is within individuals who have onset before this age.

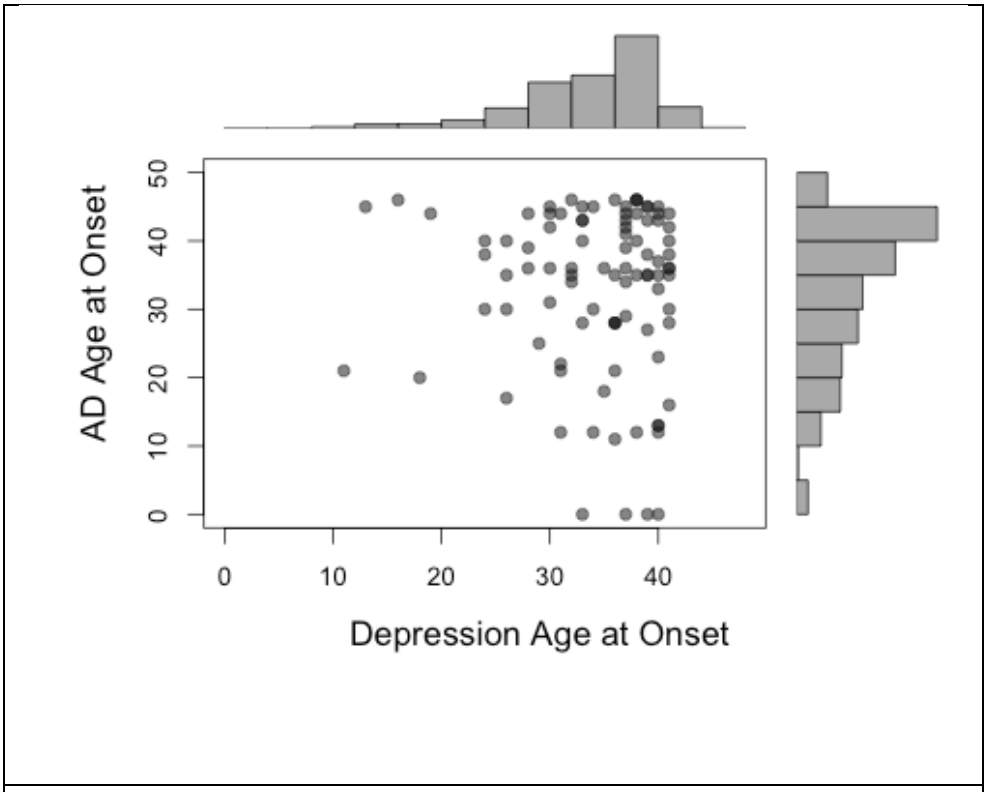

Figure 1: Age at onset distributions for depression and any autoimmune disorder (AD). Participants with both disorders are shown as points, with darker points representing more individuals with this pair of ages at onset. Of 6 participants reporting age at onset for autoimmune disorders before age 10, 4 report celiac disease and two report polyarthritis

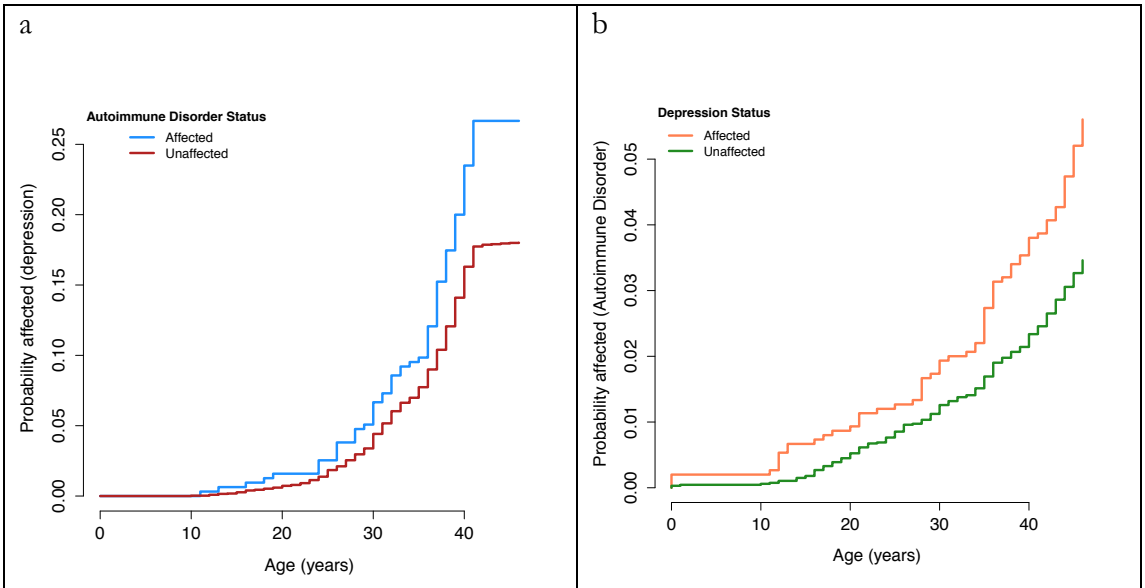

Figure 2: Curves illustrating (a) age of onset of depression by reported autoimmune disorder status, and (b) age at onset for autoimmune disorders by reported depression status. These are Kaplan-Meier curves modified to show 1 - survival

Supplementary Materials

Supplementary 1: Assessing evidence for non-random dropout

We verified that attrition and data cleaning was not introducing bias into our sample. We obtained data at waves 0 – 7, harmonised based on identification number and performed the cleaning procedure as described in methods. In this sample, we investigated a number of variables as predictors of presence versus absence of individuals in a cleaned analytic sample using a multivariate logistic regression framework.

We selected a number of biomedical predictors recorded at wave 3 (age 16) in order to test whether attrition is independent with respect to our outcome measures – these are 'Seen a Psychiatrist or Psychologist for Depression', and some proxies for autoimmune disease – Diabetes, Psoriasis and Bowel Problems (the closest proxy to IBD available in the waves 0-3 data). We also selected a number of demographic variables – gender, general ability measured at age 11 and father's social class.

	Estimate	Std. Error	z value	Pr(>|z|)
Intercept	-1.03	0.10	-9.86	$6.26x10^{-23}$
General Ability Score	0.02	0.00	18.88	$1.68x10^{-79}$
Psoriasis	-0.19	0.22	-0.86	0.39
Diabetes	0.38	0.54	0.71	0.48
Bowel Problems	-0.09	0.12	-0.78	0.44
Seen a psychiatrist for Depression	-0.05	0.33	-0.14	0.89
Female	0.22	0.04	6.06	$1.38x10^{-9}$
Father's social class II	0.03	0.09	0.33	0.74
Father's social class III	0.04	0.10	0.38	0.70
Father's social class IV	-0.08	0.09	-0.93	0.35
Father's social class V	-0.10	0.09	-1.03	0.30
Father's social class VI	-0.22	0.11	-1.97	0.05
Father's social class VII	-0.32	0.12	-2.80	0.01

Table S1: Predictors of attrition at wave 7 following data cleaning

As expected from many prior studies, low general ability score in childhood, female gender, and low paternal social class did predict attrition (Matthews et al 2006). Importantly for the current analyses, however, the medical disorders were not significant predictors of attrition. These results indicate that attrition is random with respect to our outcome measures.

Supplementary 2: Prevalence of Autoimmune Disorders

Below we have summarised the numbers of reported Autoimmune Disorders investigated in our sample, with data censored at age 46.

Autoimmune Disorder	Number of Cases	ICD-10 Code
Polyarthritis	163	M13
Rheumatoid Arthritis (Seronegative)	37	M06
Crohn's Disease	26	K50
Multiple Sclerosis	25	G35
Psoriasis	24	L40
Ulcerative Colitis	12	K51
Celiac Disease	11	K90
Type 1 Diabetes	5	E10
Sjogren Syndrome	4	M35
Pernicious Anemia	3	D51
Graves' Disease	2	E05
Pulmonary Fibrosis	2	J84
Hashimoto's	1	E06
Scleroderma	1	L94
Addison's Disease	0	E27
Dermatomyositis	0	M33
Granulomatosis	0	M31
Haemolitic Anaemia	0	D59
Lupus	0	M32
Myasthenia Gravis	0	G70
Myocarditis	0	I40
Rheumatoid Arthritis (Seropositive)	0	M05
Thrombocytopenia Purpura	0	D69
Vitiligo	0	L80

Table S2: Number of cases for each autoimmune disorder investigated, with ICD-10 code used for classification

Supplementary 3: Determining age at onset of depression

As outlined in the Methods section, we drew on reports from 3 study sweeps (waves 5, 6 and 7) to identify age at onset of depression. Not unexpectedly, we identified a small number of discrepancies in reporting across sweeps, and dealt with them as follows: Participants reporting a history of depression at wave 5 (age 33), but not at wave 6 (age 42) were excluded. Participants reporting depression at waves 5 and 6, but with reported ages at onset that differed by more than 10 years were removed. Participants with no reported onset at wave 5, but reported onset at wave 6 before age 23 were removed. Any individuals reporting age at onset for depression before age 7 were reassigned onset at age 7.

As a final step, we used 9 items from the Malaise Inventory, a tool to assess low mood (Rutter et al, 1970), to validate self-reported depression at wave 5 (Malaise items 2, 3, 5, 9, 12, 14, 16, 20, 21). We removed any cases who scored zero on the Malaise Inventory data collected at age 33, but who reported depression onset between ages 30 – 36.

Supplementary 4: Preparing Data for Rheumatoid Arthritis Polygenic Risk Score

In order to remove the effect of the WTCCC1 controls from the Okada et al (2014) GWAS, we first clean the WTCCC1 RA cases and controls using the following protocol. We first remove SNPs with minor allele frequency below 10% and Hardy-Weinberg Equilibrium *P*-value below 0.05, and individuals with missingness above 10%. We then remove SNPs with a genotyping rate below 99.5%. Finally, individuals with missingness above 1% on the remaining SNPs are removed. Following this protocol, 297,733 SNPs remain, and GWAS is performed on these cases and controls using logistic regression under an additive model.

After merging with the Okada et al GWAS, we remove SNPs with an average posterior call rate (as determined by Chiamo) > 99.9%, and ambiguous SNPs (i.e. A/T and C/G). This leads to a total of 178,239 SNPs.

We remove the effect of this GWAS using the formula below. This is a re-arrangement of the standard inverse variance weighted fixed effects meta-analysis formula, as presented elsewhere (Borenstein et al., 2009).

Where a full GWAS contains *only* effects from GWAS A and B, and:

β_{FULL} is effect size from full GWAS

β_{A} is effect size from GWAS A

β_{B} is effect size from GWAS B

SE_{FULL} is standard error from full GWAS

SE_{A} is standard error from GWAS A

SE_{B} is standard error from GWAS B

$$SE_B = \sqrt{\frac{{SE_{FULL}}^2}{1 - {SE_{FULL}}^2 / {SE_A}^2}}$$

$$\beta_B = {SE_B}^2 \left(\beta_{FULL} \left(\frac{1}{{SE_A}^2} + \frac{1}{{SE_B}^2} \right) - \frac{\beta_A}{{SE_A}^2} \right)$$

Supplementary 5: Repeating Main Analysis with Cluster Robust Standard Errors

In order to investigate the possible effect of longitudinal dependence of observations biasing our results, we repeat the main analyses here, accounting for any longitudinal dependence using cluster robust standard errors implemented in the survival package in R. The results are presented below as table S3, and show that omitting cluster robust standard errors in our main analysis biased estimates towards the null, therefore providing support for our conclusions. This is an expected result when calculating cluster robust standard errors for predictors with a negative intraclass correlation – this will be the case in our data, where individuals only contribute multiple rows if their disease status changes across the study period, thus inducing a negative intraclass correlation in these variables.

		Main Analysis		Using Cluster Robust Standard Errors	
		HR	*P*-value	HR	*P*-value
Predicting Depression Onset	Autoimmune Disorder (unadjusted)	1.39	3.71×10^{-3}	1.39	3.16×10^{-3}
	Autoimmune disorder (adjusted for Depression PRS)	1.31	0.046	1.31	0.044
	Depression PRS (adjusted for Autoimmune Disorder)	1.08	8.73×10^{-3}	1.08	8.17×10^{-3}
Predicting Autoimmune Disorder Onset	Depression (unadjusted)	1.40	9.55×10^{-3}	1.40	6.65×10^{-3}
	Depression (adjusted for Autoimmune Disorder PRS)	1.31	0.08	1.31	0.069
	Autoimmune Disorder PRS (adjusted for Depression)	1.15	0.03	1.15	0.041

Table S3: Comparison of using cluster robust standard errors with the main analysis presented above.

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Chapter 5: The Relationship between Mental Health, Disease Severity and Genetic Risk for Depression in Early Rheumatoid Arthritis

THE RELATIONSHIP BETWEEN MENTAL HEALTH, DISEASE SEVERITY AND GENETIC RISK FOR DEPRESSION IN EARLY RHEUMATOID ARTHRITIS

Running Title: "Depression in Early Rheumatoid Arthritis"

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ABSTRACT

Introduction

Reduced mental health is prevalent in rheumatoid arthritis (RA). Although longitudinal studies are limited, there is evidence that depression associates with worse disease outcomes. We evaluated reciprocal relationships between mental health, RA severity and genetic risks for depression over 2-years in a well-characterised cohort of RA patients.

Methods

We evaluated 520 early RA patients previously enrolled to two clinical trials. Mental health was measured using the SF-36 mental health (MH) domain and mental component summary scores (MCS). MCS/MH associations over two-years with disease activity (DAS28), disability (HAQ), pain visual analogue scale (VAS) scores, and a weighted genetic risk score (wGRS) for depression, were tested using linear mixed-effects and regression models.

Results

Poorer mental health associated with worse RA outcomes. Lower MCS scores (indicating worse mental health) were seen in patients with a greater genetic risk for depression (wGRS β=-1.21; *P*=0.013). Lower baseline MCS associated with lower 2-year improvements in DAS28 (β=-0.02; *P*<0.001), pain (β=-0.33; *P*<0.001), and HAQ (β=-0.01; *P*=0.006). Baseline MCS associated with changes in the swollen joint count (β=-0.09; *P*<0.001) and patient global assessment (β=-0.28; *P*<0.001), but not the tender joint count (*P*=0.983) and erythrocyte sedimentation rate (*P*=0.973). Only baseline pain VAS (β=-0.07; *P*=0.002) associated with 2-year changes in MCS.

Conclusions

Reduced baseline mental health associated with lower improvements in disease activity, disability, and pain over two years, supporting current national guidelines recommending screening for depression in RA. Pain had a bidirectional relationship with mental health. Depression genetic risk had a significant association with mental health.

Keywords

Rheumatoid arthritis; mental health; disease activity; disability; pain; genetics.

LIST OF ABBREVIATIONS

CARDERA: Combination Anti-Rheumatic Drugs in Early RA; CBT: cognitive-behavioural therapy; DAS28: disease activity score on a 28-joint count; ESR: erythrocyte sedimentation rate; GWAS: genome-wide association study; HAQ: Health Assessment Questionnaire; HWE: Hardy-Weinberg equilibrium; LD: linkage disequilibrium; LOCF: last-observation carried forward; MAF: minor allele frequency; MCS: mental component summary score; MDD: major depressive disorder; MH: mental health; NICE: National Institute for Health and Care Excellence; PCS: physical component summary score; PGA: patient global assessment; P_T: *P*-value threshold; QC: quality control; RA: rheumatoid arthritis; RCT: randomised controlled trial; REC: Research Ethics Committee; SF-36: short form-36; SJC: swollen joint count; SNP: single nucleotide polymorphism; TJC: tender joint count; VAS: visual analogue scale; wGRS: weighted genetic risk score.

1. INTRODUCTION

Reduced mental health is prevalent in rheumatoid arthritis (RA), with major depression present in 16.8% of patients [1]. The cause of this excess burden of mental health impairment is uncertain. Comorbid depression also appears to have a detrimental impact on the disease course of RA, being associated with increased healthcare utilisation and costs [2] and representing an independent risk factor for non-suicide related mortality [3]. Determining the cause and effect of depression in RA is, therefore, a key research goal.

Research in this area has mainly involved cross-sectional studies in patients with long-standing RA. These identified associations between depression, and pain [4], disability [5] and arthritis disease activity [6]. Their cross-sectional nature, however, made it impossible to infer causality. Whilst longitudinal studies are limited, there is some evidence for a bidirectional effect with pain in patients with musculoskeletal disorders, whereby depression influences pain and vice versa [7, 8]. There is also some evidence that depression influences the subsequent disease activity of RA, with an analysis of established RA patients finding a slower rate of decline in disease activity over time in patients with a history of depression [9].

Depression also has a substantial genetic component [10], with a recent mega-analysis of genome-wide association studies (GWASs) identifying multiple markers of suggestive association with major depressive disorder (MDD) [11]. The role of these in determining mental health in RA has not previously been evaluated.

The aim of our study was to evaluate the relationship between mental health and disease activity, disability, pain, and genetic risk for depression over 2 years in a well-characterised clinical trial cohort of patients with early RA. The direction of any associations was tested by

examining the impact of baseline mental health on changes in disease activity, disability and pain, and vice versa.

2. METHODS

2.1 Participants

We studied patients in the Combination Anti-Rheumatic Drugs in Early RA (CARDERA) cohort. It has been described in detail previously [12]. In brief, it comprises European ancestry RA patients enrolled to two multicentre randomised controlled trials (RCTs), CARDERA-1 and CARDERA-2 [13, 14]. Both recruited patients with early RA (<2 years duration) and active disease defined as three of ≥3 swollen joints, ≥6 tender joints, ≥45-min morning stiffness, or erythrocyte sedimentation rate (ESR) ≥28 mm/h. CARDERA-1 recruited patients between 2000-2002; CARDERA-2 recruited patients between 2003-2010. CARDERA-1 randomised patients to receive either (1) methotrexate; (2) methotrexate and ciclosporin; (3) methotrexate and prednisolone; (4) methotrexate, ciclosporin and prednisolone. CARDERA-2 randomised patients to receive either (1) methotrexate or (2) methotrexate and anakinra. Follow-up was 2 years. The current analysis is restricted to the 520 patients with baseline mental health data available.

2.2 Disease Outcomes

The following disease outcomes were captured. Firstly, disease activity (how active a patient's arthritis is) was recorded using the disease activity score with 28-joint counts (DAS28). This composite score combines information on the number of swollen and tender joints (assessed by a clinician from 28 joint counts), the patient global assessment of disease activity (PGA, which involves a patient rating their overall disease activity on a 100mm visual analogue scale) and the erythrocyte sedimentation rate (ESR) in a mathematical

formula to give an assessment of RA activity ranging from 0 to 10. Lower scores indicate less active disease, with scores >5.1, <3.2 and <2.6 indicating high disease activity, low disease activity, and remission, respectively. Secondly, disability was recorded using the health assessment questionnaire (HAQ), a patient-completed questionnaire giving a score of function ranging from 0 to 3. HAQ scores of <1, 1-2, and >2 indicate mild, moderate, and severe disability, respectively. Thirdly, pain was recorded using a 100mm patient completed pain visual analogue scale (VAS). Fourthly, health-related quality of life (HRQoL) was recorded using the short form-36 (SF-36), which is described in detail below. In CARDERA-1 the aforementioned outcomes were captured every 6-months. In CARDERA-2 they were captured at 0, 6, 12 and 24 months.

2.3 Mental Health

The SF-36 is a generic measure of health status, capturing HRQoL across 8 domains (four physical and four mental) [15]. These domains are scored 0-100, with higher scores indicating better HRQoL. They can be normalised, z-transformed and combined into mental and physical component summary scores (MCS and PCS, respectively) providing summary measures of overall mental and physical health, relative to a population mean score of 50 (SD 10) [16].

We used the mental health (MH) domain score and MCS as measures of mental health in our analysis. Both have been used to screen for depression, with an MCS cut-off of 42 having a sensitivity and specificity of 74% and 81%, respectively for detecting depressive disorder [17]. They also associate with depression severity, both cross-sectionally and over time [18].

2.4 Genotyping

CARDERA patients were genotyped on the Illumina ImmunoChip array (described in detail previously [12]). Single nucleotide polymorphism (SNP) markers were removed that had >5% missingness, were duplicates, were not in Hardy-Weinberg equilibrium (HWE; $P<0.00001$), and had a minor allele frequency (MAF) <0.01. From 196,524 pre-QC markers, 138,873 were available in the final dataset. Imputation was subsequently performed using IMPUTE2 [19] and the 1,000 Genomes Phase 1 integrated variant version 3 (March 2012) reference panel (variants filtered with a European MAF <0.01). Post-imputation SNPs were removed with low INFO scores (<0.7), MAF<0.05, HWE $P<0.000001$ and genotyping rate < 0.1, resulting in 429,193 available markers.

2.5 Genetic Risk for Depression

The recent Psychiatric Genomics Consortium MDD GWAS mega-analysis failed to find a locus of genome-wide significance, likely reflecting limited power caused by the genetic architecture of MDD (small effect sizes of individual genetic variants) and the high prevalence of MDD, which increases the difficulty in recruiting large samples of screened, low risk controls [11]. We therefore tested a weighted genetic risk score (wGRS) combining loci of nominal association with MDD for an association with mental health in CARDERA. This approach is commonly used in studies of common polygenic disorders, whose genetic architecture comprises thousands of very small effect common alleles [20, 21]. We linkage disequilibrium (LD) pruned SNPs and used a *P*-value threshold (P_T) to include SNPs in the wGRS with an MDD GWAS *P*-value below 0.05, representing nominal association. A continuous wGRS based on MDD GWAS results has been shown to predict depression in independent cohorts, with a P_T of 0.05 demonstrated to generate a wGRS with the greatest effect on MDD risk [22]. After LD pruning and thresholding, 3,010 SNPs were included in

the wGRS. The wGRS was generated for each individual in CARDERA by calculating the number of nominally associated risk alleles they carried, weighted by the log odds ratio (OR) from the MDD mega-analysis, summed across SNPs.

2.6 Statistical Analysis

2.6.1 Associations with Mental Health

Two different modelling approaches were used to evaluate the relationship between mental health, RA severity measures and genetic risk for depression. The first approach established whether mental health was associated with either RA severity measures or the wGRS for depressive disorder over time. This used a linear mixed-effects model, which incorporated either MCS or MH measured at each time-point as the response variable, regressed on the corresponding predictors (wGRS, DAS28 and its components, HAQ, or pain VAS) from each time-point. The inclusion of random effects of individual and time accounted for the within-individual correlation structure of these variables over time. The following variables were tested for their associations with MCS: age, gender, disease duration, and rheumatoid factor (RF) status. Of these, only gender improved the model fit and was included as a covariate (Supplementary Tables A.1 and A.2). The wGRS was standardised to a z-score, in order to provide interpretable β-values. Examination of residuals from a model containing time, gender and treatment only confirmed a good model fit (Supplementary Figure A.1), and variance inflation factors calculated for each predictor ensured multi-collinearity between RA outcomes and DAS28 components was not an issue (Supplementary Table A.3).

The second approach evaluated the direction of associations between mental health and RA severity, by testing if mental health at study baseline associated with 2-year changes in RA outcomes over time, or vice versa. This used linear regression models to look at the association between a) baseline MCS or MH and 2-year changes in RA severity measures,

and b) baseline RA severity measures and 2-year changes in mental health scores. These models included the baseline response variable score, treatment and gender as covariates. Examination of model residuals confirmed good model fits (Supplementary Figure A.1).

2.6.2 Missing Data Imputation

In the original CARDERA-1 trial missing data at each time-point had previously been imputed using last-observation carried forward (LOCF) analysis for study end-points (DAS28, HAQ and SF-36). In the original trial an observed case analysis had excluded a significant impact of the LOCF assumption [13]. In the original CARDERA-2 trial missing data were not imputed. For consistency in the current analysis we imputed missing, previously non-imputed CARDERA-1 data (SJC, TJC, ESR, PGA, pain VAS) and missing CARDERA-2 data using LOCF. The largest amount of missing data was seen for pain VAS (11% of observations missing across all time-points). We repeated our analysis with non-imputed data only; this excluded a significant impact of the LOCF assumption (Supplementary Table A.4).

2.6.3 Statistical Software

Analyses were performed in the statistical environment R (R Foundation for Statistical Computing, Vienna, Austria), PRSice (version 1.2) [23], IMPUTE2 [19] and PLINK (version 1.9) [24].

2.7 Ethics, Consent and Permissions

CARDERA-1 (South Thames Multi Centre Research Ethics Committee (REC) reference: MREC (1) 99/04) and CARDERA-2 (South East REC reference: MREC 02/1/089) were

ethically approved. Approval was obtained to genotype archived DNA (NRES Committee East of England – Essex REC reference: 11/EE/0544). All patients provided consent.

3. RESULTS

3.1 Patient Baseline Characteristics

Most patients were female (69%; Table 1) and RF-positive (67%). Baseline disease activity was high (mean DAS28 5.88), disability moderate (mean HAQ 1.56) and disease duration short (mean duration 3.3 months). Baseline mental health was reduced relative to the general population (mean MCS score 40.6, which is 9.4 units lower than the general population mean).

3.2 Disease Severity Associations with Mental Health

In a gender and treatment adjusted linear mixed-effects model, DAS28 (P<0.001), HAQ (P<0.001) and pain VAS (P<0.001) significantly associated with MCS (Table 2). On average over two years MCS scores were 2.22, 6.07 and 0.14 units lower per unit increase in DAS28, HAQ and pain VAS scores, respectively. This indicates that over time, the higher a patient's disease activity, disability and pain levels, the worse their mental health. In multivariate models all three disease severity measures retained a highly significant association with MCS (Table 2): HAQ (β=-3.88; P<0.001), DAS28 (β=-0.91; P<0.001), pain VAS (β=-0.05; P<0.001). Similar associations were seen with the MH domain.

3.3 MDD Genetic Risk Score Associations with Mental Health

A significant association was seen between the wGRS for depression and MCS (P=0.013) and MH (P=0.041) (Table 2). The association with MCS (P=0.033) but not MH (P=0.080) was retained in multivariate models including DAS28, HAQ and pain VAS as covariates.

Higher wGRS scores, which indicate a greater genetic risk for depression, associated with worse mental health (lower MCS and MH scores) over time (MCS β=-1.21; MH β=-1.37). Repeating the analysis with a linear mixed-effects model that incorporated a wGRS*time interaction term provided some evidence that genetic risk for depression also influenced the rate at which mental health improved over time, with a significant association seen between the wGRS*time term and MH (*P*=0.039; β=-0.83) but not MCS (*P*=0.330; β=-0.30).

3.4 DAS28 Component Associations with Mental Health

In a gender and treatment adjusted linear mixed-effects model all four DAS28 components – SJC, TJC, ESR and patient global assessment (PGA) – associated with MCS and MH scores when tested individually (Table 2). Higher scores in each DAS28 component associated with lower MCS and MH scores; this indicates that more active disease is linked with poorer mental health. On average over two years MCS scores were 0.32, 0.07, 0.13 and 0.27 units lower per unit increase in SJC, ESR, PGA, and TJC scores, respectively. In multivariate models including all 4 DAS28 components the TJC failed to retain a significant association with MCS (*P*=0.461) and MH (*P*=0.519).

3.5 Direction of Association between RA Outcomes and Mental Health

3.5.1 Association between Baseline Disease Severity and Changes in Mental Health

The only baseline RA severity measure that had a significant association with two-year changes in both MCS and MH scores was pain VAS (Table 3). Higher baseline pain VAS scores (indicating greater levels of pain) associated with lesser increases in MCS and MH scores (indicating lower improvements in mental health over time). The increase in MCS was 0.07 units less per 1mm increase in baseline pain VAS. A significant association between the

baseline TJC and 2-year changes in MH domain scores was also seen (P=0.023), although this variable did not significantly associate with 2-year changes in MCS scores (P=0.122).

3.5.2 Association between Baseline Mental Health and Changes in RA Outcomes

Baseline MCS and MH domain scores had significant inverse associations with two-year changes in DAS28 (MCS and MH P<0.001), pain VAS (MCS and MH P<0.001) and HAQ (MCS P=0.006; MH P=0.008) (Table 3). Lower baseline MCS and MH scores (indicating poorer mental health) associated with lesser improvements in DAS28, pain VAS, and HAQ scores over time. The effect sizes were, however, modest: per 10 unit increase in baseline MCS, the two-year reductions in DAS28, HAQ and pain VAS were 0.20, 0.10 and 3.30 units greater, respectively (Table 3).

Dividing patients into octiles based on their baseline MCS and plotting the mean disease severity measure in each octile demonstrated the effect of baseline MCS on RA outcomes (Figure 1). Trends towards a) worse disease outcomes at each time point and b) lower improvements in disease outcomes over 2-years across increasing baseline MCS octiles were seen (Figure 1). Over two years, mean DAS28, HAQ and pain VAS scores changed by -1.14, -0.23 and -8.11 units, respectively in the lowest MCS octile (group 1) and -1.94, -0.49 and -18.49 units, respectively in the highest MCS octile (group 8).

Examining individual DAS28 components revealed that baseline MCS and MH scores had significant inverse associations with two-year changes in the SJC (MCS and MH P<0.001) and PGA (MCS and MH P<0.001) but not the TJC (MCS P=0.983; MH P=0.226) and ESR (MCS P=0.973; MH P=0.355) (Table 3). This differential impact on DAS28 components is shown in Figure 2. Over two years, mean SJC, PGA, TJC and ESR levels changed by -0.17,

-13.91, -8.02 and -11.98 units, respectively in the lowest MCS octile (group 1) and -4.69, -20.46, -5.97 and -11.03, respectively in the highest baseline MCS octile (group 8).

4. DISCUSSION

Our study evaluated the relationship between mental health and disease activity, disability, pain, and genetic risk for depression over 2 years in a well-characterised clinical trial cohort of patients with early RA. It has three key findings. The first, and most clinically important, is that low mental health associated with poorer disease outcomes. In a repeated measures analysis, lower MCS and MH scores had significant associations with more active disease, increased disability and greater pain over two years; as MCS and MH scores increased over time DAS28, HAQ and pain levels fell. Lower baseline MCS and MH scores (indicating worse mental health) associated with a reduced improvement in disease activity and disability, suggesting that depression influences the degree to which RA improves over time. The relationship between pain and mental health appeared bidirectional, with baseline pain associating with lower improvement in MCS and MH domain scores and vice versa; this is in keeping with existing studies of musculoskeletal disorders [7, 8].

The second finding was that swollen, but not tender joint counts had a significant association with reduced mental health. In a multivariate model incorporating all four DAS28 components, the TJC failed to retain a significant association with MCS and MH scores over time. In established RA patients attending routine clinics the opposite relationship appears true, with an analysis of the CORRONA registry reporting that a lifetime depression history associated with slower improvements in the TJC but not the SJC [9]. One explanation for the lack of association between MCS/MH scores and the TJC in CARDERA is that the short

disease duration of patients means the pain pathway sensitisation characterising fibromyalgic RA − which could be particularly influenced by mental health − is yet to occur. An explanation for the association observed between MCS/MH scores and the SJC is that overlapping pro-inflammatory cytokines, which are present in high levels in early active RA, play important roles in mediating both reduced mental health and RA activity. Whilst this hypothesis is supported by evidence that administering IL-1β and TNF-α induces depressive behaviour in mice [25] and that these cytokines are elevated in the serum of depressed patients [26, 27], it fails to explain why baseline mental health scores did not predict changes in ESR levels. Further research is required in other early active RA cohorts to confirm the generalisability of our results.

Our third finding was that genetic risk for MDD had a significant influence on mental health. Although a recent large MDD GWAS mega-analysis (including 18,759 subjects) failed to find a locus of genome-wide significance, the authors' noted that their study lacked power to identify individual genetic risk variants given the genetic architecture of this common disease [11]. We therefore tested a wGRS combining 3,010 SNPs of nominal association with MDD in the GWAS for its association with mental health in CARDERA. Whilst a significant association with lower MCS and MH scores was observed, it was substantially weaker than that seen with non-genetic factors. These findings support the notion that depression is a complex disorder with a modest, albeit important, genetic contribution comprising thousands of alleles of a small effect size.

Our study replicates existing research that depression and pain have a bidirectional relationship. In CARDERA, baseline MCS and MH scores predicted two-year changes in pain VAS and vice versa. This finding has been documented in psoriatic arthritis, with

Husted et al identifying a small bidirectional relationship between MCS and pain in 394 patients followed up for a mean of 7.5 years [7]. It has also been reported in patients with persistent back, hip or knee pain [8], back pain [28, 29] and pain from a variety of disorders [30]. The complex nature of pain makes it difficult to discern mechanisms by which this pain-depression bidirectional relationship could occur. Possible mechanisms include: (1) low mood could impact on pain through promoting maladaptive coping strategies, especially catastrophizing (perceiving a situation to be worse than it is) [31]; (2) pain could impact on mental health through reducing daily activities [32] and reducing social activities [33]; (3) imbalances in shared neurotransmitters (serotonin and norepinephrine) in affective and nociceptive pathways could contribute to both mood and pain [32]. Further research is required to better characterise the mechanisms underlying this complex relationship.

Supporting our finding that mental health predicts disease outcomes over time, other studies have reported a detrimental impact of reduced mental health on DAS28-defined anti-TNF responses [34, 35]. This effect is highly relevant to stratified medicine in RA. Although in CARDERA, the impact of baseline MCS on improvements in disease outcomes over two-years was modest, if considered alongside other poor prognostic markers, such as ACPA status [36], HLA variants [37], smoking and gender [38], it could provide clinically-useful prognostic information, guiding decisions on treatment intensity and facilitating a stratified approach to managing early RA patients.

Our study has several strengths. These include its large size, recruitment from multiple centres spanning two clinical trials, the measurement of multiple disease outcomes in a highly standardised manner, and the short disease duration of RA (mean 3.3 months) leaving it well placed to examine the effects of mental health in very early disease. It also has several

weaknesses. As a secondary post-hoc analysis of existing RCTs, it did not test a pre-specified hypothesis according to a pre-determined analysis plan. It evaluated a clinical trial cohort of severe RA patients, limiting its generalisability to patients seen in routine clinical practice. Additionally, we only evaluated European ancestry individuals; the relevance of our findings to other ethnic populations is uncertain.

Current National Institute for Health and Care Excellence (NICE) guidelines for RA management recognise the importance of assessing for co-morbid depression, recommending this as part of an annual review process [39]. Our findings strongly support this recommendation in early RA. One unresolved issue is the impact of treating depression on the disease course of RA. Whilst we did not evaluate the impact of mental health therapies on RA outcomes, there is some evidence that psychological interventions (such as cognitive-behavioural therapy (CBT), disclosure therapy and biofeedback), are useful adjunctive management tools in RA patients. Two systematic literature reviews have evaluated the evidence base for this. Astin et al reported significant pooled effect sizes for psychological interventions at reducing post-interventional pain, disability, and psychological status across 25 trials [40]. Similarly, Dissanayake et al found evidence for the efficacy of disclosure therapy and CBT with maintenance therapy across 4 and 5 studies, respectively [41]. The evidence base is, however, limited with both reviews noting that available trials had methodological limitations. Further research is required to better define the impact of specific psychological interventions at improving disease outcomes in large, well-conducted clinical trials of RA patients.

5. CONCLUSIONS

In this cohort of 520 early, active RA patients reduced mental health (captured using the SF-36) associated with worse disease outcomes. Lower MCS and MH scores (indicating poorer mental health) significantly associated with more active disease, increased disability and greater pain over two years. Worse baseline mental health associated with lesser improvements in RA outcome measures, suggesting that depression influences the rate at which RA improves over time. A bidirectional relationship was observed between mental health and pain, replicating existing work in musculoskeletal disorders. Depression genetic risk had a significant, albeit modest impact on mental health. Our findings support current NICE RA management guidelines recommending the annual screening of RA patients for co-morbid depression. Further research is needed to establish the impact of specific mental health management strategies on improving RA outcomes.

Table 1. CARDERA Genetics Cohort Baseline Characteristics

Characteristic		Summary Statistic
Demographic	Number (%) Female	358 (69%)
	Mean Age in Years (SD)	54.7 (12.6)
RA Specific	Mean RA Duration in Months (SD)	3.3 (4.9)
	Number (%) RF-Positive	350 (67%)
	Mean DAS28 (SD)	5.88 (1.29)
	Mean HAQ (SD)	1.56 (0.70)
Mental Health	Mean MCS (SD)	40.6 (14.1)
	Mean MH (SD)	61.0 (18.0)
Treatment	Number (%) Receiving MTX	159 (31%)
	Number (%) Receiving MTX and CIC	108 (21%)
	Number (%) Receiving MTX and Pred	102 (20%)
	Number (%) Receiving Triple Therapy	107 (21%)
	Number (%) Receiving MTX and anakinra	44 (8%)

Cohort size used in analysis = 520 patients; SD = standard deviation; MTX = methotrexate; CIC = ciclosporin; pred = prednisolone; triple therapy = MTX, CIC and pred; RF = rheumatoid factor; DAS28 = disease activity score on a 28-joint count; HAQ = health assessment questionnaire; MCS = SF-36 mental component summary score; MH = SF-36 mental health domain. A DAS28 of 5.88 indicates highly active disease. A HAQ of 1.56 indicates moderate disability. An MCS of 40.6 is 9.4 units lower than that observed in the normal population (which has a mean score of 50.0 units).

Table 2. Longitudinal Associations between Mental Health, RA Outcomes and Depression Genetic Risk Score

	Mental Component Summary Score (MCS)				Mental Health (MH) Domain Score			
	Gender and Treatment Adjusted Model		Multivariate Model*		Gender and Treatment Adjusted Model		Multivariate Model*	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
RA Outcomes								
DAS28	-2.22 (0.16)	<0.001	-0.91 (0.21)	<0.001	-2.97 (0.21)	<0.001	-1.37 (0.27)	<0.001
HAQ	-6.07 (0.40)	<0.001	-3.88 (0.48)	<0.001	-8.41 (0.53)	<0.001	-5.75 (0.63)	<0.001
Pain VAS	-0.14 (0.01)	<0.001	-0.05 (0.01)	<0.001	-0.17 (0.01)	<0.001	-0.05 (0.02)	0.007
Genetic Risk								
MDD wGRS	-1.21 (0.48)	0.013	-0.92 (0.43)	0.033	-1.37 (0.67)	0.041	-1.05 (0.60)	0.080
DAS28 Components								
SJC	-0.32 (0.04)	<0.001	-0.12 (0.04)	0.003	-0.47 (0.05)	<0.001	-0.25 (0.05)	<0.001
ESR	-0.07 (0.01)	<0.001	-0.04 (0.01)	0.003	-0.11 (0.02)	<0.001	-0.06 (0.02)	<0.001
PGA	-0.13 (0.01)	<0.001	-0.11 (0.01)	<0.001	-0.16 (0.01)	<0.001	-0.11 (0.01)	<0.001
TJC	-0.27 (0.04)	<0.001	-0.03 (0.04)	0.461	-0.34 (0.05)	<0.001	-0.03 (0.05)	0.519

DAS28 = disease activity score on a 28-joint count; HAQ = health assessment questionnaire; pain VAS = pain visual analogue scale score; MDD wGRS = weighted genetic risk score for major depressive disorder; SJC = swollen joint count; ESR = erythrocyte sedimentation rate; PGA = patient global assessment of disease activity; TJC = tender joint count. All linear mixed-effects models include gender, treatment and time as explanatory variables; * = multivariate model for RA outcomes and genetic risk also includes DAS28, HAQ, pain VAS and MDD wGRS as explanatory variables; multivariate model for DAS28 components also includes SJC, ESR, PGA and TJC as explanatory variables.

.

Table 3. Direction of Associations between Mental Health and RA Outcomes

	Mental Component Summary Score (MCS)				Mental Health (MH) Domain Score			
	Model 1: Baseline MCS Predicting Two-Year Changes in RA Outcomes		Model 2: Baseline RA Outcomes Predicting Two-Year Change in MCS		Model 1: Baseline MH Predicting Two-Year Changes in RA Outcomes		Model 2: Baseline RA Outcomes Predicting Two-Year Change in MH	
	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value	β (SE)	*P*-value
DAS28	-0.02 (0.01)	<0.001	-0.31 (0.45)	0.492	-0.01 (0.00)	<0.001	-0.83 (0.61)	0.170
SJC	-0.09 (0.02)	<0.001	0.04 (0.09)	0.625	-0.07 (0.02)	<0.001	0.06 (0.12)	0.577
ESR	0.00 (0.07)	0.973	0.03 (0.02)	0.203	-0.05 (0.06)	0.355	0.01 (0.03)	0.590
PGA	-0.28 (0.08)	<0.001	-0.03 (0.02)	0.144	-0.27 (0.06)	<0.001	-0.05 (0.03)	0.061
TJC	0.00 (0.02)	0.983	-0.12 (0.07)	0.122	-0.02 (0.02)	0.226	-0.23 (0.10)	0.023
HAQ	-0.01 (0.00)	0.006	0.50 (0.85)	0.554	0.00 (0.00)	0.008	0.19 (1.13)	0.865
Pain VAS	-0.33 (0.08)	<0.001	-0.07 (0.02)	0.002	-0.31 (0.06)	<0.001	-0.08 (0.03)	0.005

DAS28 = disease activity score on a 28-joint count; HAQ = health assessment questionnaire; pain VAS = pain visual analogue scale score; SJC = swollen joint count; ESR = erythrocyte sedimentation rate; PGA = patient global assessment of disease activity; TJC = tender joint count. All linear regression models include gender, treatment and baseline measure of response variable tested as covariates.

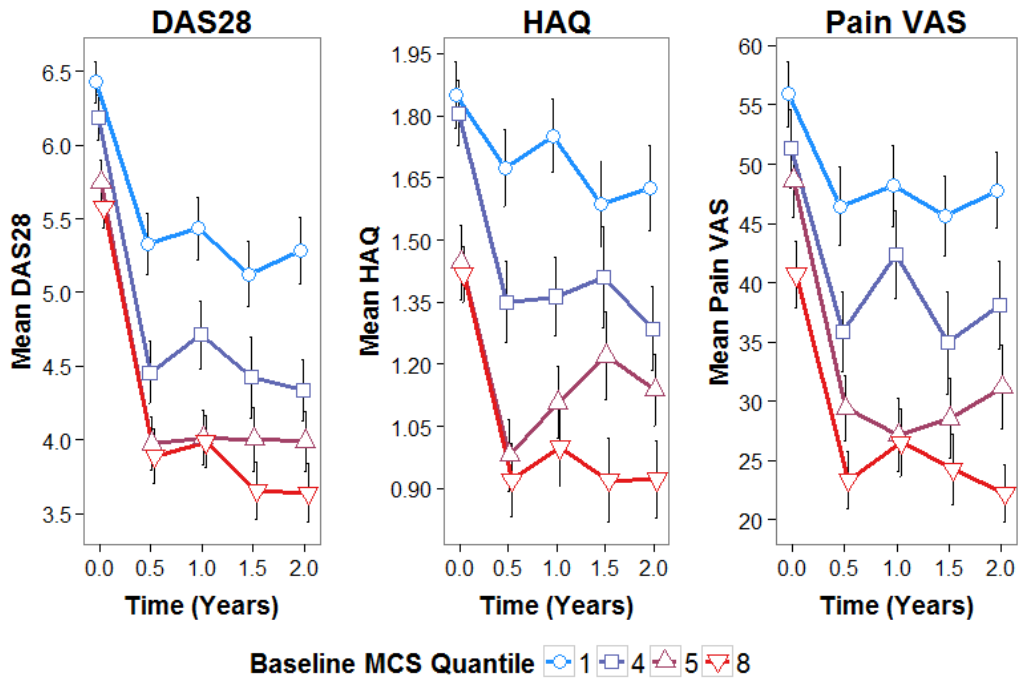

Figure 1. Mean DAS28, HAQ and Pain VAS Stratified by Baseline MCS Octile

MCS divided into octiles (8 quantiles); mean scores with standard error bars for octiles 1, 4, 5 and 8 plotted at each time point; to facilitate visual interpretation octiles 2, 3, 6 and 7 are not plotted, although the same trends are observed (Supplementary Figure 2).

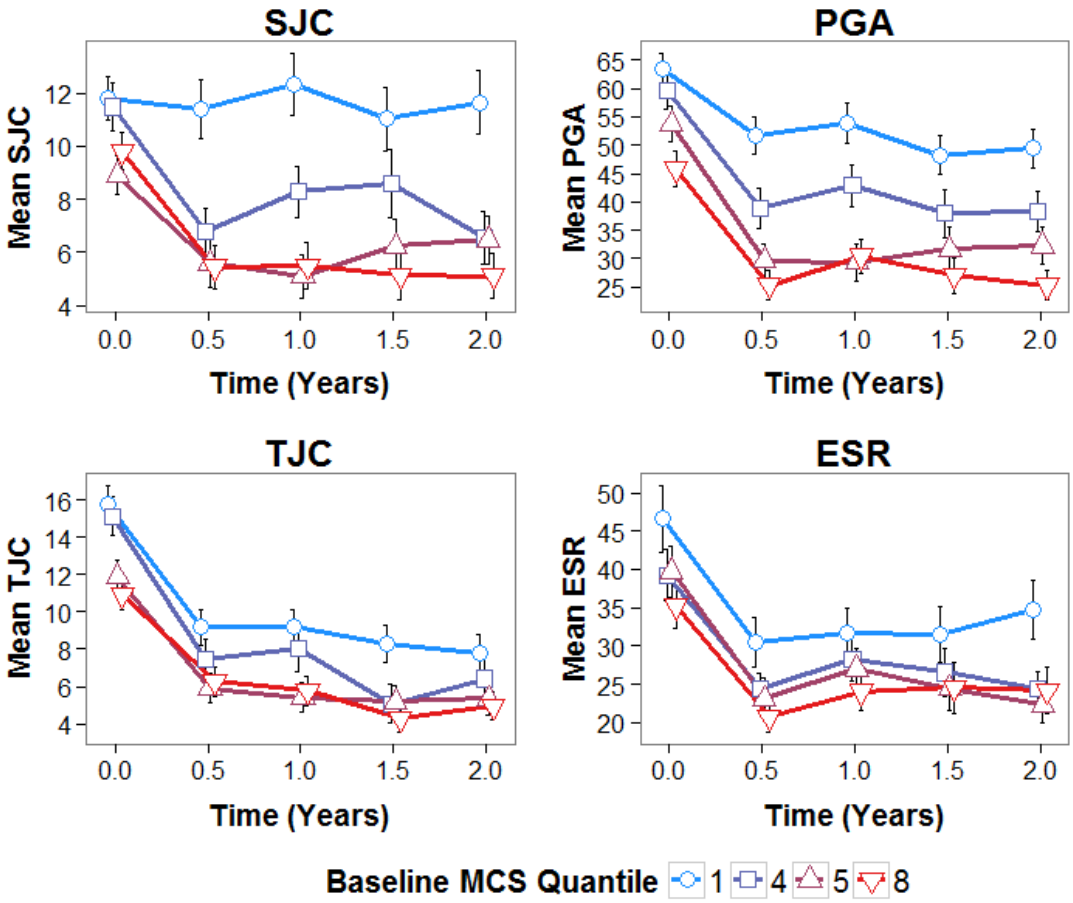

Figure 2. Mean DAS28 Components Stratified by Baseline MCS Octile

MCS divided into octiles (8 quantiles); mean scores with standard error bars for octiles 1, 4, 5 and 8 plotted at each time point; to facilitate visual interpretation octiles 2, 3, 6 and 7 are not plotted, although the same trends are observed (Supplementary Figure 3).

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THE RELATIONSHIP BETWEEN MENTAL HEALTH, DISEASE SEVERITY AND GENETIC RISK FOR DEPRESSION IN EARLY RHEUMATOID ARTHRITIS

SUPPLEMENTARY MATERIAL APPENDICES

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Table A.1. Selection of Modelling Covariates in Linear Mixed-Effects Model

Variable	Elimination Number	*P*-Value
RF-Positive	1	0.780
Disease Duration	2	0.354
Age	3	0.286
Treatment	kept	0.027
Gender	kept	0.001
Time	kept	<0.001

A stepwise AIC was used to select modelling covariates that significantly predicted MCS scores over time within a linear mixed-effects model. After three iterations, only treatment, gender and time were significant predictors of MCS and were included as modelling covariates (treatment and gender as fixed-effects, and a random effect of time).

Supplementary Table A.2. Associations between Modelling Covariates and MCS in Linear Mixed-Effects Model

Variable		β	SE	P-Value
Treatment	*Methotrexate Monotherapy*	Reference	Reference	Reference
	Ciclosporin-Methotrexate	-2.50	1.37	0.069
	Prednisolone-Methotrexate	-0.32	1.40	0.818
	Methotrexate-Prednisolone-Ciclosporin	2.27	1.37	0.099
	Methotrexate-Anakinra	1.52	1.89	0.421
Gender		3.35	1.04	0.001
Time		1.71	0.31	<0.001

Model includes MCS as response variable and treatment, gender and time as explanatory variables.

Table A.3. Variance Inflation Factors (VIF) for Predictors in Multivariate Linear Mixed-Effects Models

A: VIF for RA severity metrics

Variables	VIF
HAQ	1.55
DAS28	2.01
Pain	1.76
wGRS	1.02

Model includes MCS as response variable and treatment, gender, time, HAQ, DAS28, Pain VAS, and wGRS as explanatory variables.

B: VIF for DAS components

Variables	VIF
ESR	1.12
TJC	1.51
SJC	1.41
PGA	1.51

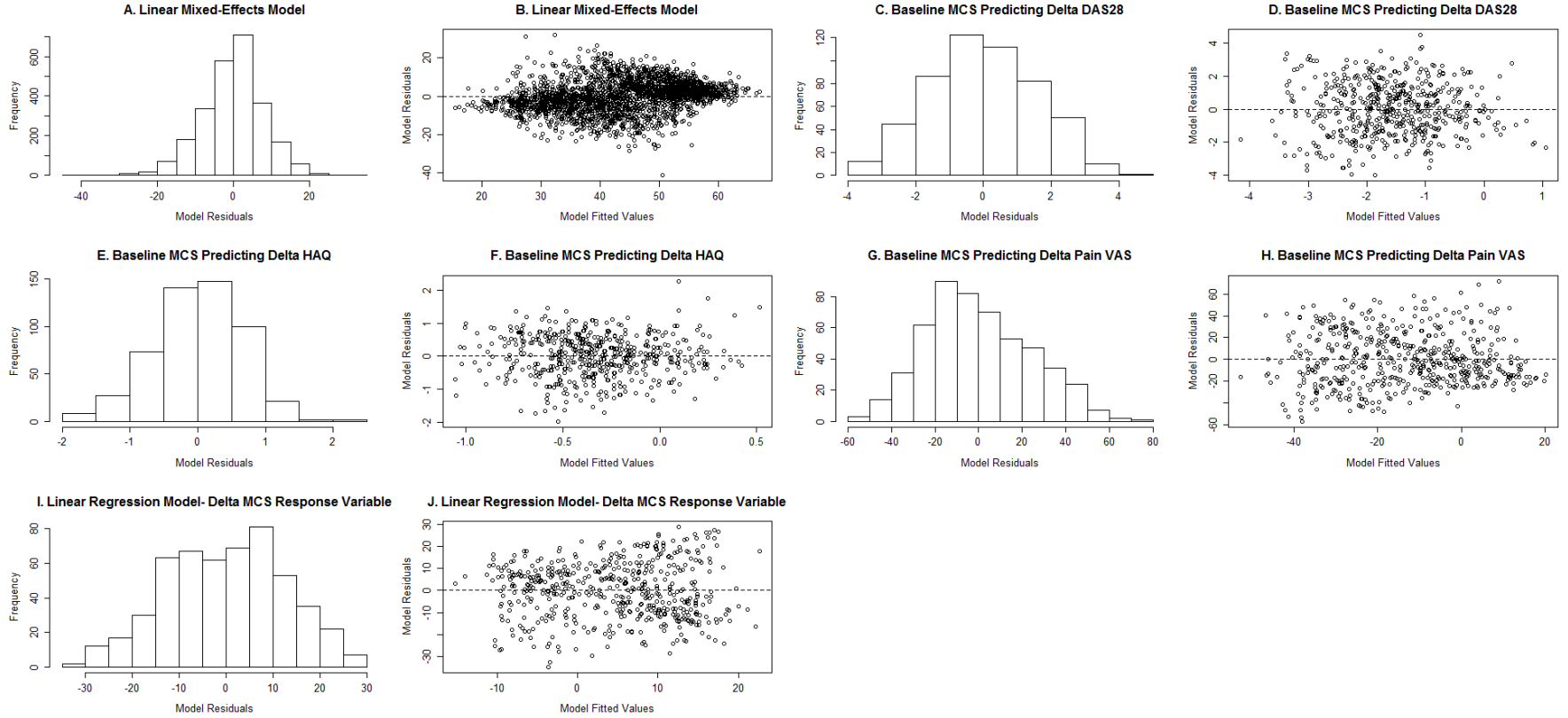
Model includes MCS as response variable and treatment, gender, time, ESR, TJC, SJC, and PGA as explanatory variables.

Table A.4. Associations With MCS Using Linear Mixed-Effects Model In Imputed Versus Non-Imputed Data

	Non-Imputed		Imputed	
Variable	**β**	***P*-value**	**β**	***P*-value**
TJC	-0.28	<0.001	-0.27	<0.001
SJC	-0.35	<0.001	-0.32	<0.001
ESR	-0.07	<0.001	-0.07	<0.001
PGA	-0.14	<0.001	-0.13	<0.001
HAQ	-6.10	<0.001	-6.07	<0.001
Pain	-0.14	<0.001	-0.14	<0.001
DAS28	-2.25	<0.001	-2.22	<0.001

To ensure LOCF imputation had not biased our results we repeated the analysis using non-imputed data. The results are highly similar, confirming the LOCF assumpation was met. A linear mixed-effects models is used in this analysis, including MCS as the response variable and time, treatment, gender and each RA outcome as an explanatory variable in seven separate models (one per RA outcome)

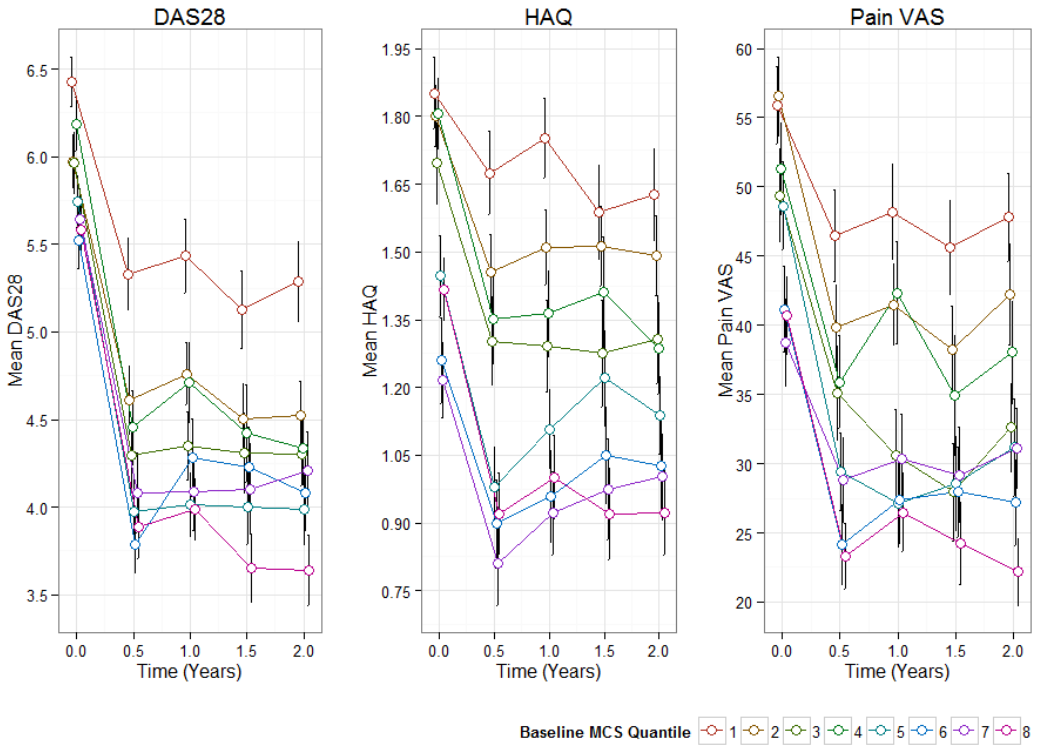
Figure A.1. Residual Plots from Linear Mixed-Effects and Linear Regression Models



Figure

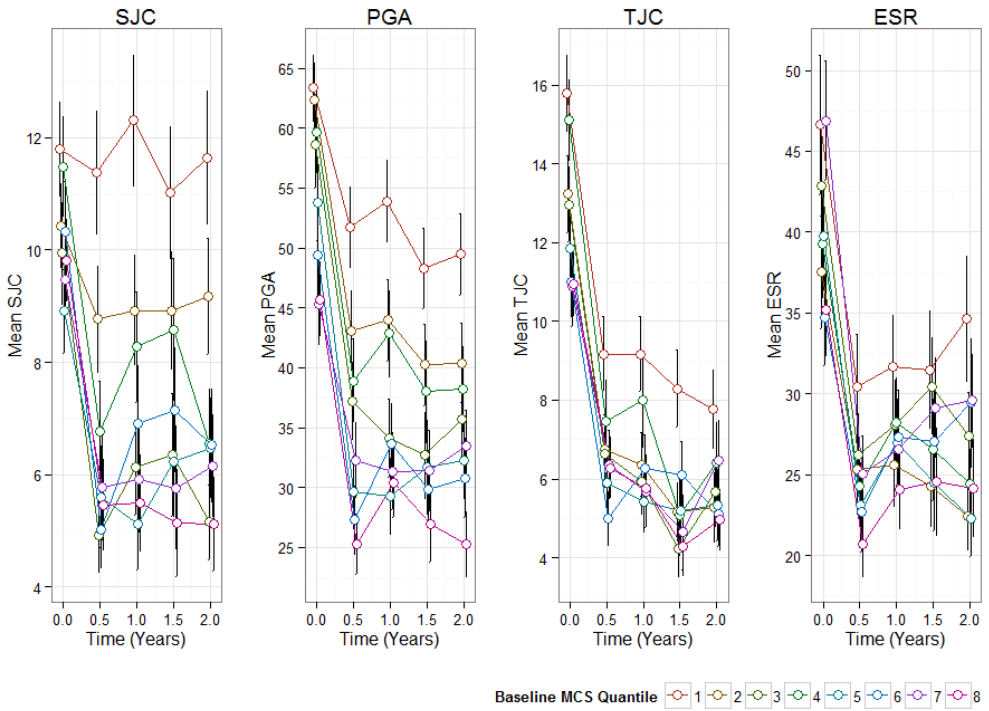
A, C, E, G and I are histograms of residuals from each model; B, D, F, H and J are plots of the fitted values against the residuals from each model; A and B = linear mixed-effects model including MCS as the response variable and gender, treatment and time as explanatory variables; C and D = linear regression model including 2-year change in DAS28 as the response variable and baseline MCS, baseline DAS28, gender and treatment as explanatory variables; E and F = linear regression model including 2-year change in HAQ as the response variable and baseline MCS, baseline HAQ, gender and treatment as explanatory variables; G and H = linear regression model including 2-year change in pain VAS as the response variable and baseline MCS, baseline pain VAS, gender and treatment as explanatory variables; I and J = linear regression model including 2-year change in MCS as response variable and baseline MCS, gender and treatment as explanatory variables.

Figure A.2. Mean DAS28, HAQ and Pain VAS Stratified by Baseline MCS Octile



MCS divided into octiles (8 quantiles); mean scores with standard error bars for octiles are plotted at each time point

Figure A.3. Mean DAS28 Components Stratified by Baseline MCS Octile



MCS divided into octiles (8 quantiles); mean scores with standard error bars for octiles are plotted at each time poin

Chapter 6: PRSlice: A localised Polygenic Risk Score method

PRSlice: A localised Polygenic Risk Score method for cross-trait analyses

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Abstract

Biomarkers, biologically derived variables that have utility in predicting disease, are sought-after in many medical fields. Here we present a novel method for using DNA to calculate a biomarker for genetic risk of disease, building on the established method of Polygenic Risk Scoring. Our novel method outperforms conventional Polygenic Risk Scoring approaches when using the genetic risk for one disease as a predictor of another correlated trait. We propose that this method will have growing utility as the diversity of phenotypes interrogated using complex disease genetics methods increases. We validate our method through application to real data, and produce simulated data in order to interrogate the performance of the method under well-characterised scenarios of genetic overlap between pairs of correlated complex phenotypes.

Introduction

Over the last five years, Polygenic Risk Scoring (PRS) has emerged as a dominant strategy for calculating genetic risk of a disorder in samples of genotyped individuals and has already been exploited across a range of applications (Dudbridge, 2013; International Schizophrenia Consortium et al., 2009; Power et al., 2015; Power et al., 2014; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). In addition to identifying evidence for shared genetic aetiology between traits, PRS are valuable as variables to model the genetic component of disease risk, and can be used to predict disease risk and proxy the genetic contribution to liability within large epidemiological cohorts – as discussed in chapter 4 - or to explore the more subtle aspects of the genetic architecture of a trait, such as gene-environment interactions (Mullins et al., 2016; Peyrot et al., 2014).

The standard PRS approach, as developed by Purcell et al (2009), has been widely applied with relatively little modification, in a diversity of scenarios. The aggregate effect of risk variants, as identified by a 'base' Genome-Wide Association Study (GWAS) are tested on phenotype in an independent genotyped sample termed the 'target' data. Variants are obtained in approximate linkage equilibrium by performing 'linkage-disequilibrium (LD)-informed clumping' on the base data using LD information from the target data. This allows SNP effects in the base data to be considered to be additive. This 'clumped' base GWAS is used to prioritise variants with nominal association with the base phenotype, by ranking variants by *P*-value and selecting those below some *P*-value threshold – usually denoted P_T. A Polygenic Risk Score, PRS, is calculated at this threshold P_T for each individual in the target data by summing each risk allele – that is, alleles with $P<P_T$ in the base GWAS – weighted by its effect size in the base GWAS – that is, beta – for a continuous outcome - or the natural logarithm of its odds ratio - for a binary outcome. PRS for each individual in the target data is then regressed on the target phenotype, to test the prediction of the PRS. This is iterated across a number of values for P_T in order to determine the most predictive score, as performed by PRSice (Euesden, Lewis & O'Reilly, 2015), which can then be used for further analysis.

More broadly, the aim of understanding the genetic architecture across traits has given rise to a number of methods – notably, LDpred and LD Score Regression. These

methods vary in both their requirements in terms of input and also in the interpretation of their results. LDpred (Vilhjalmsson et al., 2015) is developed from the assertion that the LD-informed clumping described above may be redundant as, given a plausible model for the expected distribution of causal effect sizes underlying observed GWAS results – such as an infinitesimal model – it is possible to shrink observed GWAS effect sizes, accounting for LD, in order to obtain GWAS effects to be considered independent and thus additive. The authors propose combining all SNPs genome-wide, weighted by these shrunken effect sizes, to produce a more accurate PRS, and demonstrate its performance in a number of real data and simulation-based scenarios, however the method is yet to be widely used. Secondly, LD Score Regression (LDSC) (Bulik-Sullivan et al., 2015), is a method that relies on a rearrangement of the formulae used for calculating heritability from kinship matrices (Yang, Lee, Goddard, & Visscher, 2011) to enable the same calculations to be performed on GWAS summary statistics in combination with an appropriate LD matrix. This method has an advantage over PRS in that it can be performed entirely using data that is typically released publically following the publication of a GWAS, however as it does not use individual-level data, it cannot be used to stratify at an individual level or predict risk, and has mainly been used to date to identify evidence for genetic overlap between pairs of disorders from GWAS summary data. Thus data availability and study aim may determine the preferred methodology for investigating genetic overlap.

As the application of PRS across scientific questions widens, its underlying methodology will require modification in order to address different questions optimally. Here we introduce one such modification for a particular application of polygenic risk scoring – that of the polygenic risk score as a biomarker. In several cases within complex disease genetics, researchers may wish to calculate a variable for individuals' genetic risk of a trait, for example Major Depressive Disorder (Psychiatric GWAS Consortium et al., 2013). However, this score may be underpowered; both because of heterogeneity in MDD and because even the largest published GWAS to date on MDD are based on relatively small sample sizes. We propose that it is possible to leverage our knowledge that MDD shares a number of risk alleles with Schizophrenia (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013; Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013; Euesden et al., 2015) – which has been explored far more thoroughly by higher powered GWAS (Schizophrenia Working Group of the

Psychiatric Genomics Consortium, 2014) – and so use this information to calculate a better predictor for our phenotype of interest, in this example MDD.

Materials and Methods

Method

First we split the genome into chunks, each 5 Mb in length. For each chunk, PRS is calculated in the normal way, calculating scores at a large number of thresholds, regressing each on phenotype in the target data, and selecting the score at the best threshold. This approach allows the most predictive threshold for each region to be selected, allowing a large number of variants to be included from some regions and few from others.

PRS at all chunks are then sorted based on their *P*-value for association with the target phenotype. Scores are calculated by adding one chunk at a time, in ascending order of *P*-value and testing the predictive value of each aggregated score on the target phenotype. The sum of chunks that maximally predicts target phenotype is identified and used as a new PRS tailored for predicting across traits that share genetic architecture. This protocol is outlined graphically in Figure 1, and as pseudocode in supplementary 3.

Due to the large multiple testing burden implicit in this analysis, we propose calculating an appropriate alpha threshold empirically. This is achieved by running the above method over a large number of iterations, permuting case-control (or quantitative trait phenotype data) in the target data, and using the test statistic from regression of the most predictive score predicting target phenotype at each iteration to generate a null test-statistic distribution.

This method – which we call PRSlice – makes use of pre-existing bioinformatics software developed myself, PRSice (Euesden et al., 2015), which incorporates PLINK2 (Chang et al., 2015). We also distribute software to reproduce our analysis protocol here, currently available on request as PRSlice_v0.02, which is optimised to run on a Sun Grid Engine. All other statistical analyses outlined here are performed in R version 3.2.2.

We hypothesise that PRSlice will outperform PRSice in situations where genetic correlation between traits is modest but not complete. PRSlice enables risk loci specific to a single phenotype to be discarded from the final risk score, whilst regions that do appear to have an effect to both phenotypes are retained. This has the effect of removing noise from the calculation of the new risk score. By contrast, as genetic correlation tends towards one, we hypothesise that the improvement due to PRSlice - which discards regions associated with base phenotype but not target phenotype – will be weaker as fewer regions are associated with base phenotype but not target phenotype.

Application to real data

We apply PRSice and PRSlice to 24 real data scenarios to compare their relative performance in predicting one phenotype using the PRS of another. These scenarios correspond to all possible combinations of 4 target phenotypes – MDD in the RADIANT and UK biobank samples, BMI and Ever-smoked in the Northern Finland Birth Cohort data – and 6 base phenotypes – PGC2 Schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), PGC1 MDD (Psychiatric GWAS Consortium et al., 2013), Global Lipids Consortium High-Density Lipids (HDL) and Low-Density Lipids (LDL) (Global Lipids Genetics Consortium et al., 2013), GIANT Consortium BMI (Locke et al., 2015) and Tobacco and Genetics (TAG) Consortium 'Ever Smoked' (Thorgeirsson et al., 2013). The effect of the NFBC individuals, who are present in the GAINT consortium data, are removed using a method described elsewhere (Chapter 4, supplementary 4). We remove the effect of the RADIANT study from the PGC MDD GWAS by meta-analysing the other 8 PGC1-MDD studies, as described elsewhere (Mullins et al., 2014).

Details on Target Data Sets

The RADIANT Major Depressive Disorder (MDD) study (Lewis et al., 2010) is a case-control data set comprising 1,624 cases and 1,588 controls. Cases are drawn from three studies focussing on recurrent MDD. Controls are psychiatrically screened. All cases and controls have been genotyped on the Illumina Human610-Quad BeadChip.

The Northern Finland Birth Cohort comprises a population cohort collected from individuals born in 1966 in Oulu, Northern Finland (Rantakallio, 1988). Of this sample, 5402 individuals have been both genotyped on the Illumina Infinium 370cnvDuo array (Sovio et al., 2009), and phenotyped – however, completeness of phenotyping varies from measure to measure.

The UK Biobank (UKBB) sample used here is from the first wave of genotyping released from this cohort, consisting of 117,310 individuals after quality control. This sample has been phenotyped on a wide range of measures, however under the limits of our current data application, we investigate depression exclusively as a target phenotype. Individuals were phenotyped for depression using the criteria described elsewhere (Smith et al., 2013). This sample was genotyped on two Affymetrix microarrays, the UK BiLEVE and UKB Axiom arrays. Genotype data is imputed to a combined reference panel of UK10K and 1000Genomes. Full data on quality control procedures are available (http://www.ukbiobank.ac.uk/wp-content/uploads/2014/04/UKBiobank_genotyping_QC_documentation-web.pdf).

Determination of Significance Threshold

To determine the appropriate significance threshold likely resulting from a degree of overfitting, we first permute case-control status 100,000 times in the case of schizophrenia predicting MDD, calculating model fit of most predictive score at each permutation, allowing us to determine the appropriate alpha threshold to maintain a family-wise error rate of 5%. Due to the substantial running time of 100,000 permutations, we test consistency compared to 10,000 and 1000 permutations. We apply 1,000 permutations to the HDL GWAS predicting into the NFBC genotypes with phenotypes permuted.

Simulation Study

We also validate our method by comparing the improvement between prediction using our novel method to prediction calculated when using a standard approach to Polygenic Risk Scoring, using simulated data. We use HAPGEN2 (Su, Marchini, & Donnelly, 2011) to simulate genotype data under a White Western European LD structure, using the HapMap3, release 2, CEU genotypes for haplotype data (International HapMap Consortium, 2003).

We simulate base and target populations under three different genetic models. In the first, "same-trait", the genetic architecture in both the base and target data is the same. In the second, "cross-trait different effects", case, we simulate similar but non-identical genetic architectures in the base and target data sets. In the third case, "cross-trait subset of effects", case, we simulate only a subset of the effects present in the base data in the target data, reflecting only a subset of loci being pleiotropic. In each scenario, we simulate 100 Mb genomes for 20,000 individuals in a base data set (10,000 cases, 10,000 controls) and 2,000 individuals in a target data set (1,000 cases, 1,000 controls) using SNPs from HapMap3 release 2. We simulate 15 causal SNPs in the base data with effect sizes – i.e. proportion of phenotype variance explained - drawn from a distribution with parameters fixed to ensure heritability sums to a pre-specified value. Minor allele frequencies for all SNPs are derived from the HapMap data. Full details of this protocol are outlined in supplementary 2.

Phenotypes in all simulated individuals are calculated as case-control traits, under a liability threshold model, calculated in GCTA (Yang et al., 2011). These base and target data are used to run simulations. We compare the relative performance of PRSlice and PRSice in predicting phenotype in each case, using the framework outlined in supplementary 2.

In all cases, we use LD Score Regression (Bulik-Sullivan et al., 2015) to calculate the enetic correlation - r_G - between base and target samples, and test relative PRSice and PRSlice performance as a function of this genetic overlap.

Results

Real Data Applications

We apply PRSlice and PRSice to perform 24 real-data cross-trait analyses. The sample characteristics are summarised in Table 1. The results of applying the two methods to these data are summarised below (Table 2).

	Target Dataset and Phenotypes			
	RADIANT	**UKBB**	**NFBC**	
	MDD	**Depression**	**BMI**	**Ever Smoked**
N	3212	23,726	4594	4,699
N cases	1624	8,074	NA	2996
N controls	1588	15,652	NA	1703
Mean (SD)	NA	NA	24.67 (4.21)	NA

Table 1: Sample characteristics across target cohorts

We incorporate publically available data on the genetic correlation (r_G) between pairs of disorders (Bulik-Sullivan et al., 2015), estimated from publically available summary statistics. This allows us to compare the performance of PRSlice versus PRSice against estimates of the underlying genetic architecture between pairs of disorders. We would expect PRSlice to perform best when the genetic correlation between traits is relatively low. We exclude pairs of disorders for which Bulik-Sullivan et al do not report significant evidence for a genetic overlap (P>0.05), and define the relative performance of the methods as PRSlice as $-\log_{10}$(PRSlice P-value) minus $-\log_{10}$(PRSice P-value). This measure circumvents concerns about the comparability of different pairs of phenotypes, as the influence of power on differences between scenarios will be mitigated. Absolute r_G is associated in direction, but non-significantly, with reduced PRSlice power compared to PRSice (coefficient = -5.61, P=0.37).

			Target Data set and Phenotype															
			NFBC								RADIANT				UKBB			
			BMI				Ever Smoked				MDD				Depression			
			PRSice		PRSlice		PRSice		PRSlice		PRSice		PRSlice		PRSice		PRSlice	
	Sample	Phenotype	β	P	β	P	β	P	β	P	β	P	β	P	β	P	β	P
Base GWAS	GIANT consortium	BMI	11921	$7.7x10^{-52}$	13.8	$2.1 x10^{-42}$	1197	$1.0 x10^{-1}$	-4.9	$1.2 x10^{-6}$	684	$1.1 x10^{-1}$	6.1	$1.4 x10^{-9}$	NA	NA	NA	NA
	Global Lipids Consortium	HDL	-26981	$6.9 x10^{-33}$	-10.7	$3.1 x10^{-26}$	-1843	$1.0 x10^{-2}$	-5.8	$6.8 x10^{-9}$	221	$1.2 x10^{-3}$	6.1	$1.3 x10^{-9}$	-860	$3.3 x10^{-2}$	4.4	$1.3 x10^{-5}$
		LDL	15467	$1.2 x10^{-14}$	7.9	$3.4 x10^{-15}$	280	$5.7 x10^{-1}$	3.9	$8.2 x10^{-5}$	-2512	$1.7 x10^{-4}$	-4.7	$2.0 x10^{-6}$	1864	$1.6 x10^{-2}$	7.0	$1.8 x10^{-12}$
	Psychiatric Genomics Consortium	MDD	-652	$7.9 x10^{-2}$	-4.9	$1.1 x10^{-6}$	-61	$4.9 x10^{-2}$	4.5	$6.3 x10^{-6}$	1547	$7.0 x10^{-5}$	4.6	$4.4 x10^{-6}$	54	$3.0 x10^{-4}$	4.8	$1.4 x10^{-6}$
		SCZ	-1141	$3.3 x10^{-2}$	5.7	$1.4 x10^{-8}$	-80	$2.3 x10^{-1}$	4.3	$1.5 x10^{-5}$	1277	$2.0 x10^{-11}$	11.9	$8.6 x10^{-33}$	1853	$2.3 x10^{-20}$	10.3	$6.3 x10^{-25}$
	Tobacco and Genetics Consortium	TAG	614	$1.0 x10^{-2}$	4.4	$9.3 x10^{-6}$	2642	$2.6 x10^{-5}$	4.7	$2.2 x10^{-6}$	1711	$2.4 x10^{-5}$	4.6	$5.0 x10^{-6}$	NA	NA	NA	NA

Table 2: Results of applying PRSice and PRSlice to psychiatric and biometric base and target data sets. The scale of β varies between PRSice and PRSlice analyses due to differences in score calculation and is presented here to show direction of effect. Cells with $\beta < 0$ are shaded grey

3.2 Permutation Analyses

The empirical alpha threshold appropriate for controlling the family-wise error rate at 5% is broadly consistent across simulations (Table 3). Based on these results, we propose that an alpha threshold of $P<10^{-12}$ is appropriate.

Permutation scenario	Number of Permutations	Empirical Alpha Threshold for FWE = 0.05
Schizophrenia into RADIANT	1,000	$1.24x10^{-11}$
Schizophrenia into RADIANT	10,000	$9.48x10^{-12}$
Schizophrenia into RADIANT	100,000	$8.08x10^{-12}$
HDL into NFBC	1,000	$2.22x10^{-12}$

Table 3: Empirical significance thresholds determined from permutations across null data

Simulation Results

In each of our 1000 simulations of same effects in base and target trait, and 1000 simulations of different effects in base and target trait, we calculate the statistical power as the proportion of simulations in which the *P*-value for PRSice exceeds the calculated alpha empirical threshold for PRSice in that simulated data set, and the proportion of simulations in which the *P*-value for PRSlice exceeds the calculated alpha empirical threshold for PRSlice in that data set. This provides an indication of the statistical power of PRSlice compared to the standard PRS approach of PRSice.

In the first, "cross-trait same effects" case, we found that, using PRSice, power was higher for cross trait (Table 4, χ^2, $P<10^{-16}$), and when using PRSlice, power was also slightly higher for same trait (Table 4, χ^2 *P*-value=$2.6x10^{-3}$).

We consider correctly identified genetic overlap as an outcome and use logistic models to identify factors predicting this from our simulation results. In a multivariate model, both PRSlice vs PRSice (coefficient = 1.17, $P=8.2x10^{-16}$) and Same trait vs Cross-trait (coefficient = 1.35, $P<10^{-16}$) predicted improved power. A suggestively significant

negative interaction effect between these two predictors (coefficient = -0.57, P=0.079) suggests that the outperformance of PRSlice vs PRSice is larger in cross-trait scenarios.

In the second, "cross-trait different effects", case, in which the simulated target phenotype, under the cross trait case is less genetically related to the base phenotype, we again find PRSlice outperforms PRSice in the cross trait scenario (χ^2 P-value $<10^{-16}$), and again find more modest evidence for PRSlice outperforming PRSice in the same-trait scenario (χ^2 P-value = $5.1\text{x}10^{-3}$). In a multivariate model, as described above, we find PRSlice and Same-phenotype scenarios are significant predictors of higher power, however we also find substantial evidence for a PRSlice*Cross-trait interaction term (β =1.39, P = $3.2\text{x}10^{-6}$) indicating PRSlice gives higher power in cross-phenotype scenarios.

Method	**Genetic Architecture**	**Cross-trait scenario**	**Power (% correctly identified)**
PRSice	Cross Trait	Cross-trait same effects	83.7%
	Same Trait		95.9%
PRSlice	Cross Trait		95.0%
	Same Trait		98.0%
PRSice	Cross Trait	Cross-trait different effects	58.3%
	Same Trait		95.7%
PRSlice	Cross Trait		92.2%
	Same Trait		97.9%

Table 4: Relative power of PRSice and PRSlice under different simulated genetic architectures, when modelling cross-trait scenarios using different genetic effects

Incorporating r_G Estimates into Simulation Results

Due to our simulation protocol, the genetic correlation between base and target phenotype, in the correlated trait scenarios, will not be fixed across different simulated data sets, and will thus vary. We use LDSC to estimate the actual genetic correlation between base and target in each of our 2000 simulated scenarios. In some cases, due to the relatively small sample sizes of our target data sets, the estimation was underpowered, but results from data sets in which these estimators could be accurately estimated

demonstrated that in cross-trait only simulations, PRSlice predicted a substantial increase in power (coefficient = 1.85, P=1.21x10^{-10}) when adjusting for r_G. However, PRSlice had no effect vs PRSice in same-trait cases when adjusting for r_G (coefficient = 0.65, P=0.12).

When investigating the "cross-trait different effects" subset of simulation results, with a lower genetic overlap between base and target data in the cross-phenotype case, we find consistent results. PRSlice predicted a substantial increase in power (coefficient = 2.08, P<10^{-16}) when adjusting for heritability, with evidence for an interaction between 'same trait' and PRSlice predicting lower power (coefficient = -1.31, P=6.0x10^{-3}) when adjusting for heritability, indicating that PRSlice has a greater improvement in power in cross-phenotype scenarios. These results are summarised below in Figure 4).

Aggregating across all simulation results, we find base-target r_G predicts improved power (coefficient = 2.12, P<10^{-16}) as would be expected, and that when adjusting for r_G PRSlice also predicts increased power versus PRSice (coefficient = 1.74, P<10^{-16}). Furthermore, we find evidence for an interaction effect (coefficient = -0.65, P = 0.015) between using PRSlice and r_G predicting power, indicating that PRSlice may outperform PRSice at lower values of r_G.

Discussion

Polygenic Risk Scoring has a number of applications across medical and population genetics – these include, but are not limited to, identifying evidence for a polygenic signal in a GWAS (International Schizophrenia Consortium et al., 2009), identifying evidence for a shared genetic component between pairs of phenotypes (Power et al., 2015; Power et al., 2014) and as a biomarker to identify the effects of genetic risk within downstream modelling (Mullins et al., 2016; Peyrot et al., 2014). It is to this latter application that our method presented here is particularly tailored to. We have presented a novel method to improve the predictive accuracy of PRS when examining cross-phenotype scenarios.

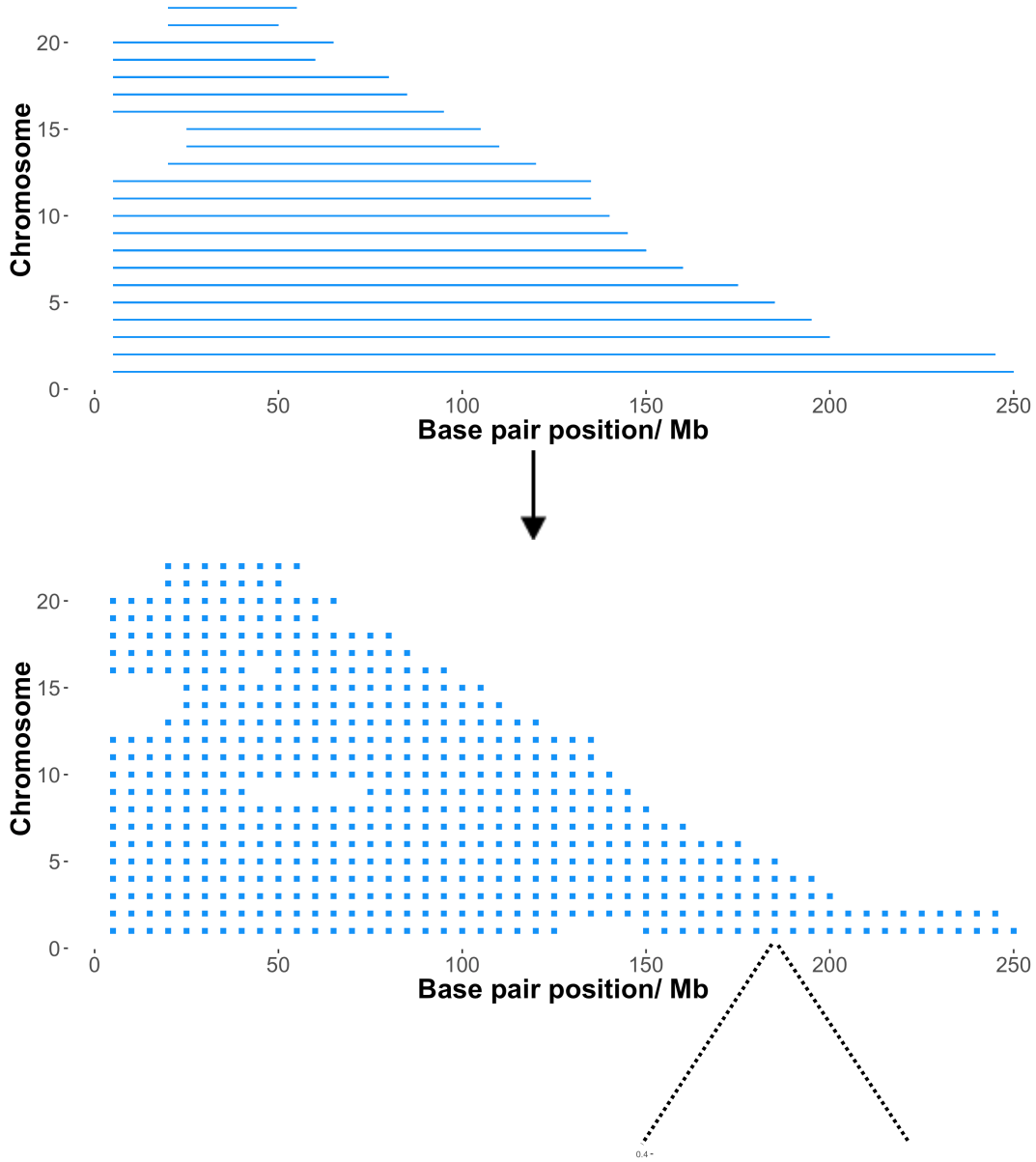
Our simulation framework demonstrates that PRSlice may outperform traditional polygenic risk scoring methods under scenarios of moderate but significant genetic overlap. By allowing the construction of PRS to account more fully for scenarios such as

allelic heterogeneity, or the preferential agglomeration of causal variants within particular regions, we are thus able to improve the predictive power of these scores. It is likely that this application will have a growing utility in the future as the asymmetry between phenotypes that have been well-studied by large genotyping efforts – such as schizophrenia or rheumatoid arthritis – and those that researchers may wish to predict in small cohorts, such as treatment response (Coleman et al., 2016), widens.

Our new method suffers from a number of limitations. Firstly, it relies on a particular contingency – a well-powered base GWAS with a modest genetic correlation to the target trait of interest; this is not immediately identifiable in many cases, and requires either pre-existing evidence from the literature, or exploratory analyses via methods such as LD Score Regression to establish that the use of PRSlice is justified. Secondly, although we have provided a proposed significance threshold of $\alpha = 10^{-12}$, the interpretation of scores generated using PRSlice is not necessarily straightforward, and thus we would recommend setting an alpha threshold via permutation for each use on different data.

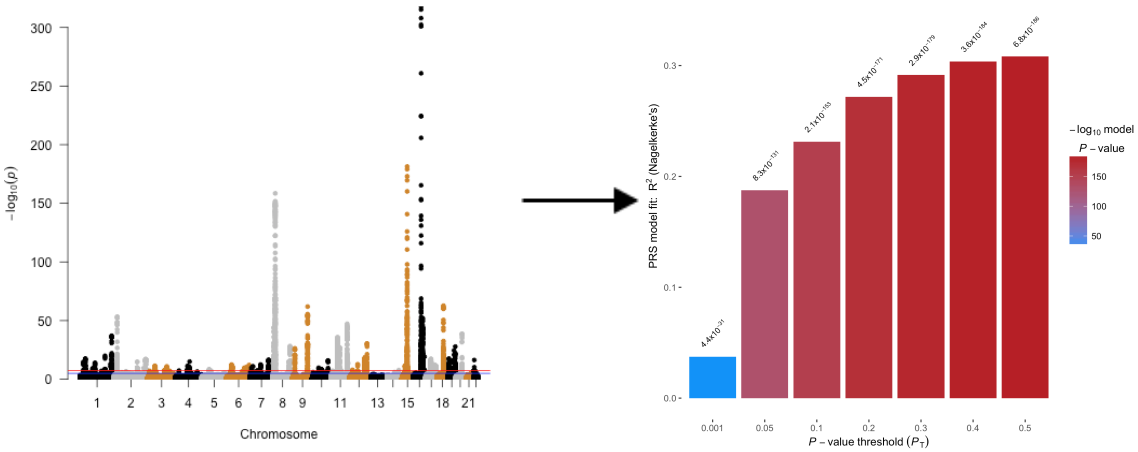
Summary

Here we present a novel method for calculating PRS, optimised for a specific scenario in which PRS is being used as a biomarker, calculated from a GWAS for a trait with a moderate genetic correlation with the target phenotype of interest. This method, PRSlice, is publically available as software, and here we present its application to real data, provide an appropriate significance threshold, and demonstrate its value using simulated data. We propose that PRSlice will have greater utility in the future as the scenario for which it is developed becomes more commonplace.



Step 1: Input base data set, cleaned for SNPs also present in target data

Step 2: Split base GWAS into 5Mb chunks using positional information



Step 3: Calculate PRS at each Chunk, using the standard approach, to obtain best threshold and P-value at this threshold

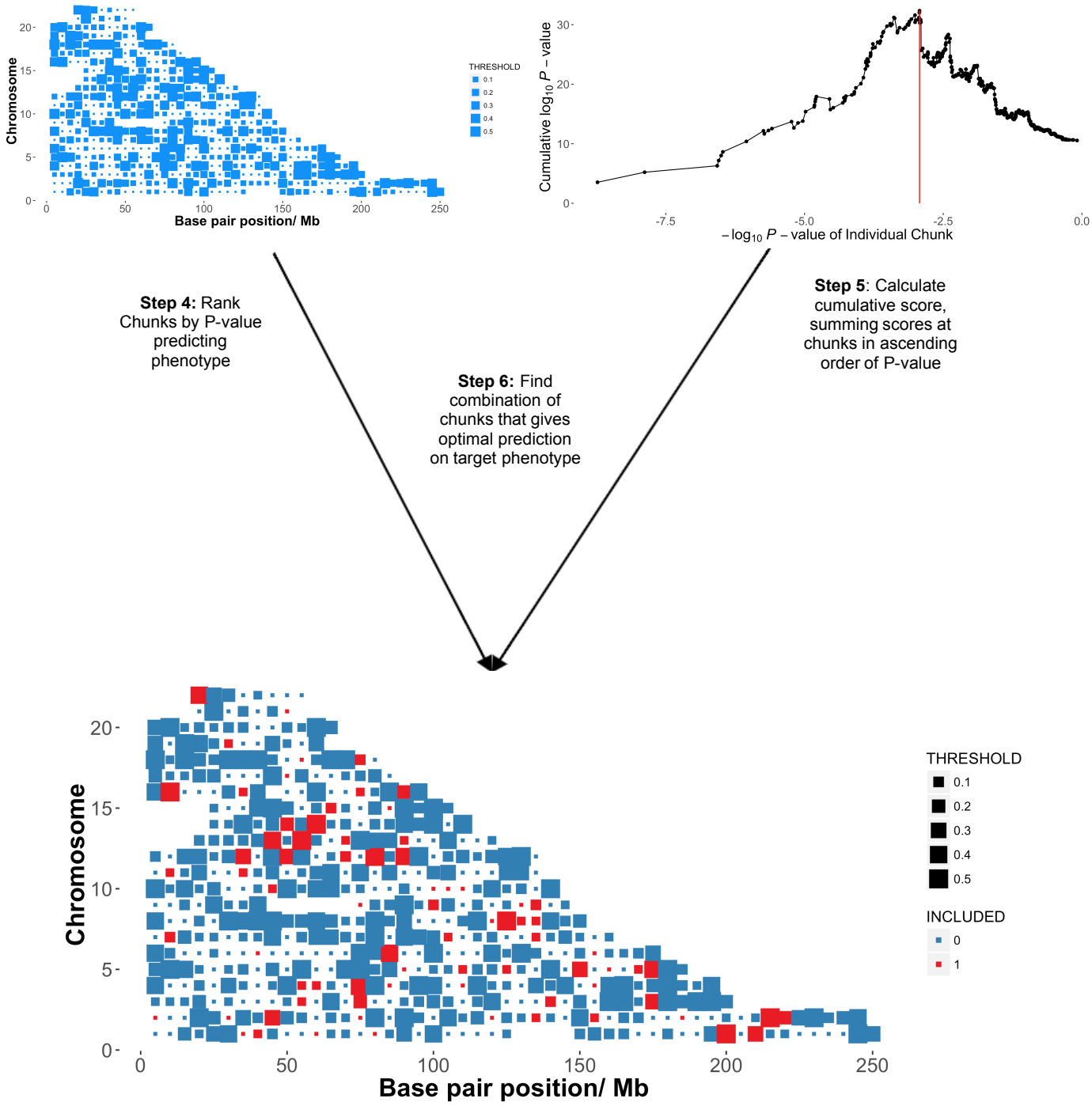
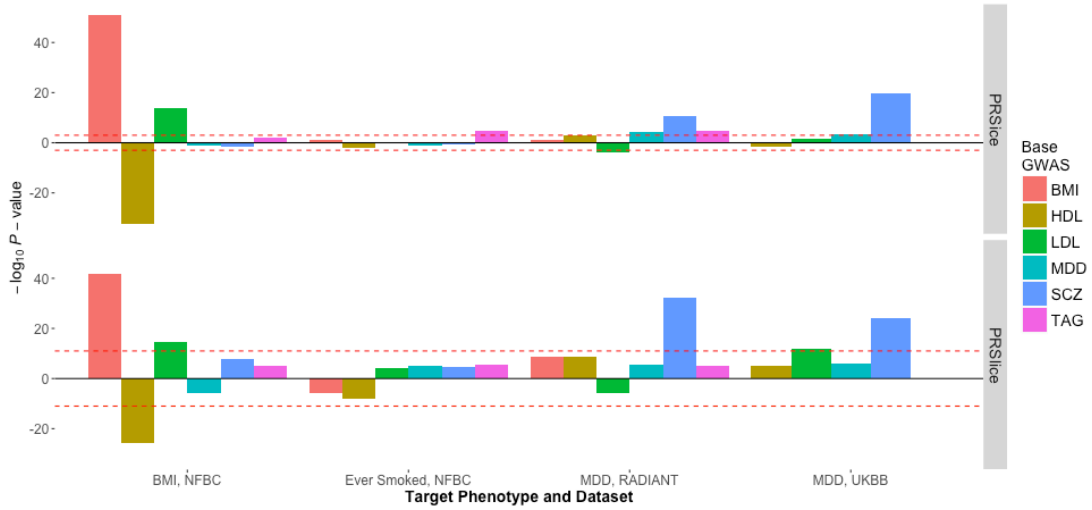


Figure 1: Protocol used for PRSlice. The genome is split into 5Mb chunks (step 2), and PRS is applied at each 5Mb chunk using the standard approach to obtain best threshold and the *P*-value with which PRS predicts phenotype at this chunk (step 3). Chunks are ranked by the *P*-value with which they predict target phenotype (step 4) and score at best threshold is summed cumulatively – the prediction of this cumulative score is tested with the addition of each chunk (step 5). The optimum combination of chunks is obtained from this, and the scores at these chunks are summed to form the final PRSlice variable (step 6)

a)



b)

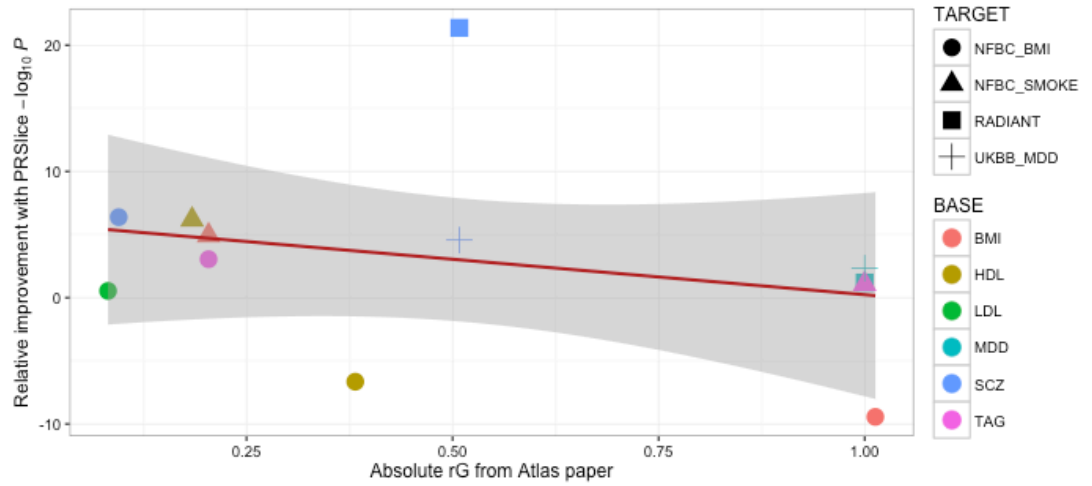
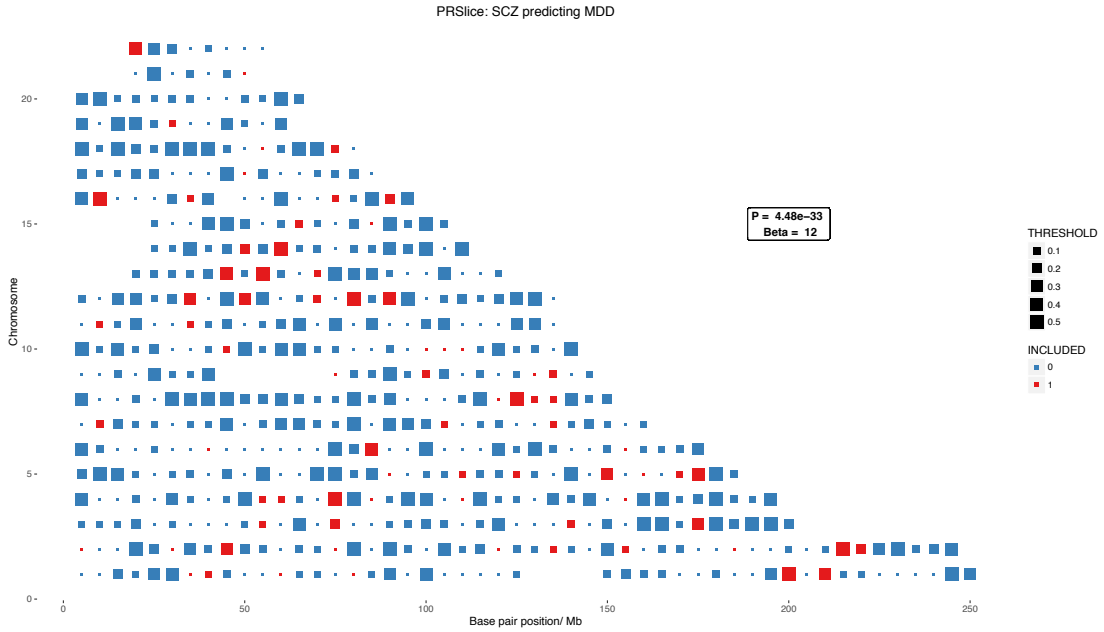
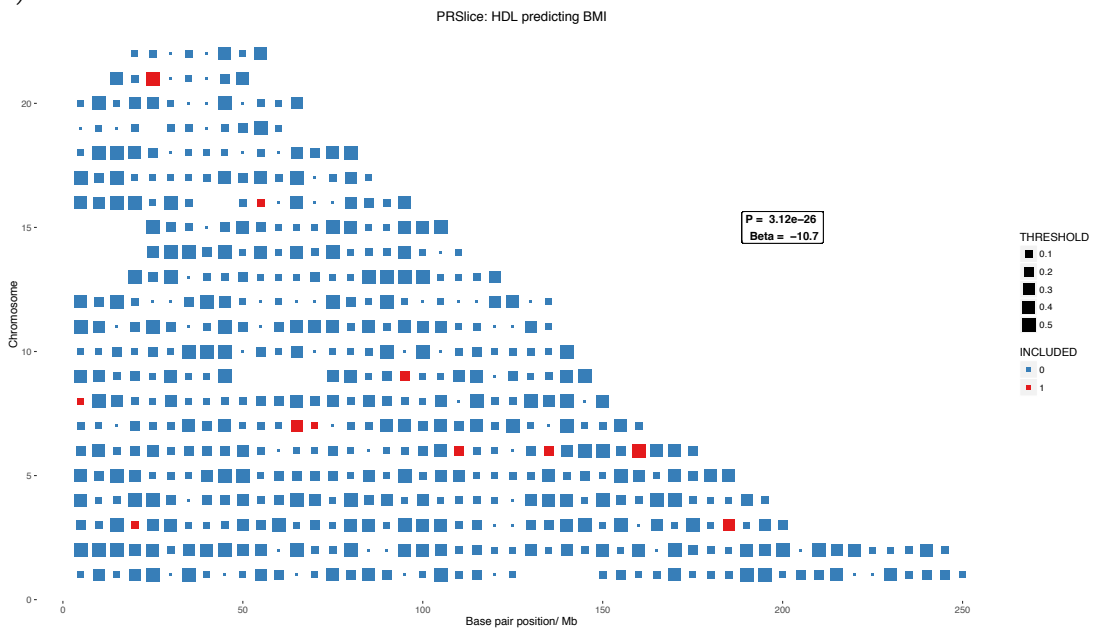


Figure 2: Real data application. a) $-\log_{10}$ *P*-values for PRSice (upper panel) and PRSlice (lower panel). Effects in the same direction in base and target are positive values on the y-axis, effects in the opposite direction are negative values on the y-axis. Significance thresholds for PRSice ($\alpha = 10^{-3}$) and PRSlice ($\alpha = 10^{-12}$) are marked in red. b) Relative performance of PRSlice over PRSice (measured as $-\log_{10}$ *P*-value for PRSlice minus $-\log_{10}$ *P*-value for PRSice) plotted against the reported genetic correlation between pairs of phenotypes as reported in Bulik-Sullivan et al (2015). Pairs of disorders that the authors do not find evidence for a significant genetic overlap between (i.e. *P*>0.05) are omitted here.

a)


PRSlice: SCZ predicting MDD
P = 4.48e−33
Beta = 12
THRESHOLD
0.1
0.2
0.3
0.4
0.5
INCLUDED
0
1
Chromosome
Base pair position/ Mb

b)


PRSlice: HDL predicting BMI
P = 3.12e−26
Beta = −10.7
THRESHOLD
0.1
0.2
0.3
0.4
0.5
INCLUDED
0
1
Chromosome
Base pair position/ Mb

c)

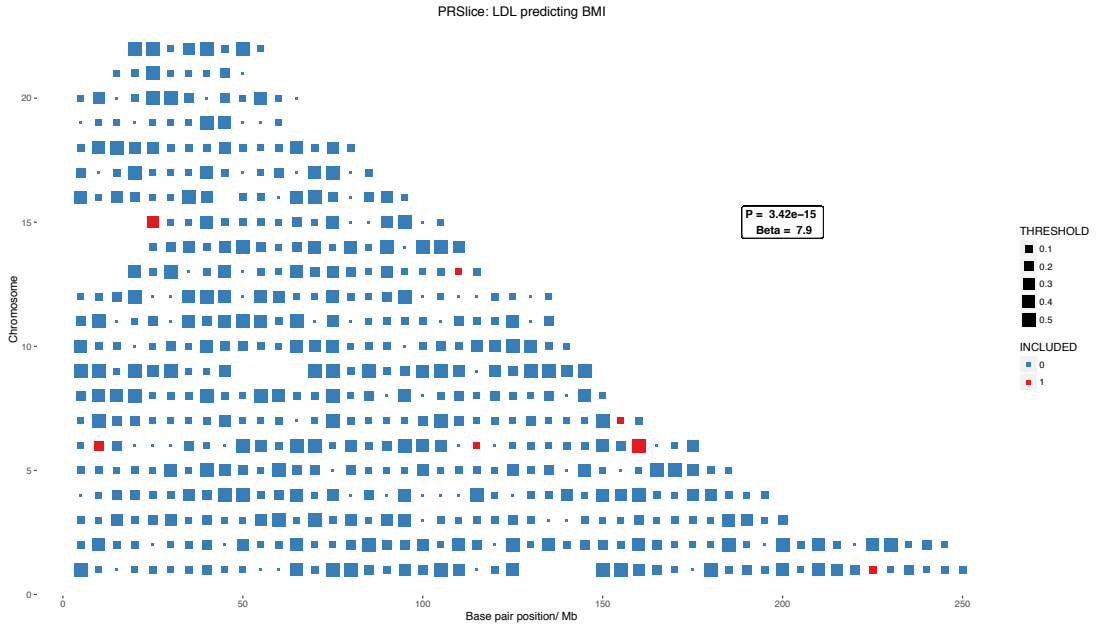
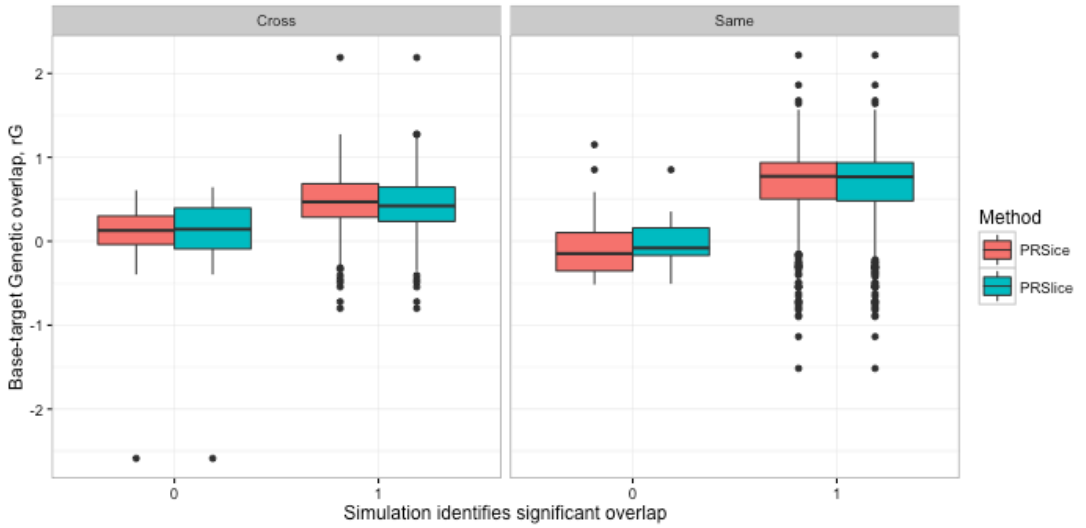


Figure 3: Selected region plots from real data application – SCZ predicting MDD in the RADIANT sample **(a)**, and both HDL **(b)** and LDL **(c)** predicting BMI in the NFBC

a)



b)

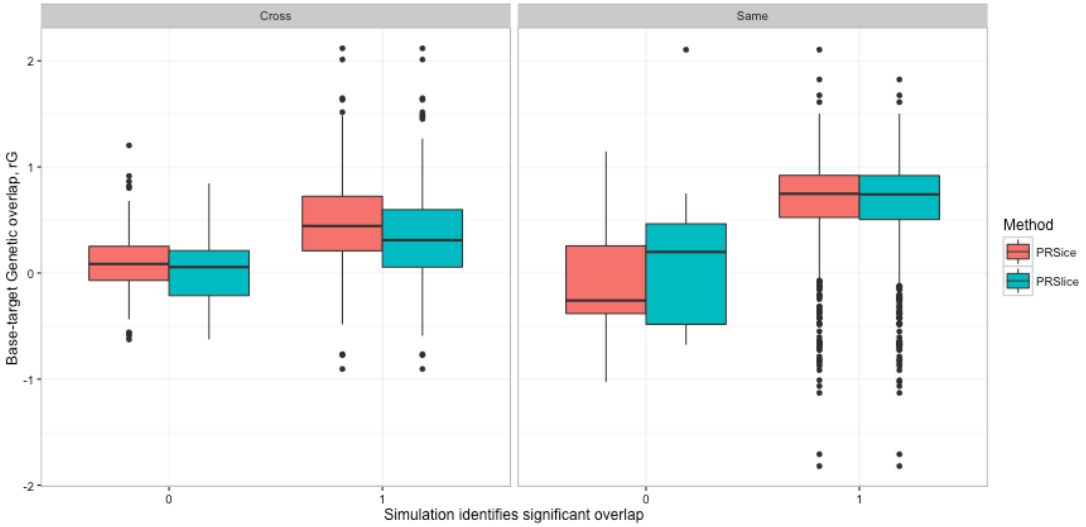


Figure 4: Performance (i.e. power) of PRSice and PRSlice in simulated data, under two scenarios - same phenotype in base and target, and cross phenotype in base and target. We present results using "cross-trait same effects" with the same five chunks causal in both cases (a) and "cross-trait different effects" with only 2/5 chunks causal in the cross-phenotype target data (b)

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Supplementary Materials

Supplementary 1: Generation of Data for Simulation Framework

We use the following simulation procedure in order to test the performance of PRSice versus PRSlice in situations with known genetic architecture. 100Mb genomes are simulated using HapMap3 reference data to simulate LD in HAPGEN2. These 100Mb genomes can be considered comprised of 20 5Mb 'chunks'.

The effect sizes of 15 causal variants are chosen by drawing from a distribution generated using the formula below with parameters fixed to ensure 15 causal variants will explain a known total of variance explained, i.e. heritability. These 15 effect sizes are split into quintiles (i.e. five groups of three) based on effect size - very low, low, intermediate, high and very high - each group of three assigned to one of five 5Mb chunks randomly selected as being causal. The remaining 75Mb of the simulated genomes do not contain causal variants. Simulated phenotypes are derived from these simulated genomes using GCTA.

We test the performance of our PRS methods under two simulated architectures. In the first architecture, effect sizes are the same for a given causal SNP in both base and target data; this simulates the effect of using a phenotype to predict the same phenotype in an independent sample. In the second architecture, the same 5 chunks are selected as containing the same causal variants in the base and target data, but the assignment of effect sizes to these SNPs - using the above categories very low, low, intermediate, high and very high - is randomised between base and target. This second architecture simulates the effect of different but correlated traits in the base and target data that show a degree of pleiotropy.

We simulate 20,000 individuals in the base data (10,000 cases, 10,000 controls) and 2,000 individuals in the target data (1,000 cases and 1,000 controls) for each of these two scenarios, fixing heritability at 15%, using the CEU European reference data from HapMap3 to model realistic haplotype information.

β_i is the effect of SNP *i* on phenotype. We fix a known number of SNPs, n, to have an effect on phenotype, defined as $c_i = 1$. These effects are defined as:

$$\beta_i = \begin{cases} 0 & if\ c_i = 0 \\ f(i,n) & if\ c_i = 1 \end{cases}$$

and

$$f(i,n) = \frac{\frac{1}{A+i}^{B}}{\sum_{i=1}^{i=n} \frac{1}{A+i}^{B}}$$

Where

A = 0.6

B = 0.8

n = 15

In the "cross-trait, different effects" subset of simulations, we simulate cross-trait data in order to reduce the genetic overlap between base and target. This may be less realistic, but the exaggerated non-overlap is intended to explore the performance of PRSlice when genetic overlap is low.

Base data is simulated with 15 causal SNPs, split evenly across 5 causal chunks, as outlined above. Of the 5 causal chunks in the base data, two chunks are selected at random to be causal in the target data. 6 effect sizes are selected from the effects in the base data to be assigned to these chunks. Thus, whilst trait heritability in the base data is 15%, the heritability in the cross-phenotype target data will vary and may be substantially lower. We then investigate the relative performance of PRSice and PRSlice in same-trait and cross-trait scenarios using the protocol outlined above.

Supplementary 2: Comparison between PRSice and PRSlice

We compare the relative performance of PRSice and PRSlice in our simulated data, generated in step 1, using the following protocol. Data is generated, the PRSice and PRSlice used to identify the most significant predictor – in the case of PRSice the most significant *P*-value threshold P_T, in the case of PRSlice the optimum thresholding across chunks and optimum combination of chunks – for a given data set. We then permute phenotype in this data set, and run PRSice, and PRSlice, 1000 times each on these permuted data sets, in order to obtain a distribution of null P-values, 1000 each for PRSice and PRSlice respectively. The observed *P*-values for PRSice and PRSlice are then tested against their respective null-distributions in order to obtain an empirical PRSice *P*-value and PRSlice *P*-value for a given simulation data set. Finally we repeat this protocol 1000 times in order to obtain 1000 simulation-derived empirical *P*-values for PRSice and 1000 simulation-derived empirical *P*-values for PRSlice.

Supplementary 3: Pseudocode Illustration of PRSlice Algorithm

```
# Step 1
Take GWAS with SNPs, genomic position of SNPs, reference
allele and P-value and OR for association with base trait – Y1

# Step 2
Divide genome into n chunks

# Step 3
for i in 1:n

  ##Step 3.1 Run standard PRS protocol on chunk i
  for j in P-value threshold values (PT) – e.g. sequence from
0-0.5 in increments of 0.001
    Calculate PRSi,j for P < j for all individuals in target
data
    Build model for PRSi,j predicting target data phenotype Y2,
glm1, Y2 ~ PRSi,j
    Calculate P-value for PRSi,j predicting Y2 in glm1
  Done

  ##Step 3.2 Determine a number of variables
  PT[i] -  The best threshold for predicting phenotype using
only SNPs in chunk i
  PvalPRS[i] – The P-value for SNPs in chunk i below PT[i]
predicting Y2
  PRSi – a vector of PRS values (i.e. PRSi,PT[i]) for every
individual in the target data
Done

We now have minimum P value - PvalPRS[i] at optimum PT for
every chunk i

# Step 4
Rank chunks by ascending order of PvalPRS and build an m x n
matrix – where there are m individuals in the target data, and
n chunks – PrsMat. Each column of PrsMat is PRS for every
individual in the target data at a different chunk, columns
are ordered by increasing values for PvalPRS

# Step 5
Produce a second m x n matrix CumPrsMat. Each column is a
cumulative sum of all previous columns, with the first column
being unchanged from PrsMat

# Step 6
for k in 1:n
  Test cumulative score – CumPrsMat[,k] – predicting Y2
  Store P-value from this model
Done

Find maximum value for k – this is the optimum combination of
chunks and thresholds
```

Chapter 7: Discussion

Epidemiological relationships, that is to say patterns of overlap or non-overlap of multiple phenotypes within individuals at rates above - or below – expectation based on their individual population distributions and prevalences, form the heart of this thesis. These relationships may frequently provide routes to understanding aetiological overlap between phenotypes, and by extension a route to understanding the pathophysiology of previously poorly understood disorders. This paradigm has a number of important steps. Firstly, one must verify that an observed epidemiological relationship is not confounded, and therefore not merely a spurious association. Secondly, one must draw inferences on the direction of causality between two epidemiologically overlapping phenotypes. Once these two have been established, and a true shared pathway between comorbid phenotypes seems likely, it is possible to leverage biological information to identify candidates for this pathway. Finally, when the biological mechanism behind a disorder has been elucidated, drug targets can be identified and novel treatment and prophylaxis investigated.

In this thesis, we have applied the above paradigm to the study of the overlap between the psychiatric and autoimmune disorders. We have used three data sources to investigate the presence and direction of comorbidity – case-control genotype data, survey and genotype data from a population-based birth cohort, and fine-scale clinical data from an hospital-based outpatient cohort. We have used existing statistical genetics techniques to investigate the evidence for overlapping pathways between pairs of disorders, and have developed a novel method to improve this approach. The conclusions of these findings will be discussed below, alongside a review of future applications of the methods we have used.

Evidence for an Epidemiological Link between Psychiatric and Autoimmune Disorders

We summarise the contents of three chapters below, in each case evaluating the strength of evidence for co-morbidity between psychiatric disorders – schizophrenia and depression – with autoimmune disorders, focussing on rheumatoid arthritis. Following this we discuss models for comorbidity consistent with our findings, and the implications of these models. Schizophrenia is arguably the best understood of the psychiatric disorders, from a genetic perspective, partly due to its higher heritability, partly due to the relative ease of phenotyping cases, and partly due to the absence of a need to screen controls, because schizophrenia has relatively low prevalence. These factors allow large case control cohorts to be collected, in turn generating more statistical power. Depression, by contrast, has been associated with the fewest genetic variants to date, however is the leading cause of disability worldwide. Thus we focus on these two very different psychiatric disorders in order to identify commonalities that might be extrapolated across the other psychiatric disorders. Rheumatoid arthritis is studied in particular as it is genetically well understood – associated with over 100 risk loci (Okada et al., 2014) – and clinically defined through a number of symptom dimensions. The poor prognosis of unmedicated rheumatoid arthritis (Fisher & Scott, 2001) also leads to increased healthcare utilisation, making the collection of detailed longitudinal data simpler than many other autoimmune disorders such as ulcerative colitis or celiac disease.

Schizophrenia

We report data on the lack of evidence for a genetic overlap between schizophrenia and rheumatoid arthritis (Euesden, Breen, et al., 2015), (Chapter 3), however over the course of the last five years, epidemiological literature on the relationship between schizophrenia and the autoimmune disorders has grown substantially. In our meta-analysis of studies published prior to November 2013, we find evidence for an epidemiological overlap between schizophrenia and rheumatoid arthritis. Furthermore, the disparate ages at onset between these two phenotypes suggest the possibility of a temporal relationship, which is often considered a precursor to establishing evidence for a causal relationship. Authors consistently find that rheumatoid arthritis is less common amongst schizophrenia patients than would be expected based on its prevalence – often termed an inverse relationship. Since the publication of our meta-analysis, Sellgren et al have performed analyses with improved granularity able to use time-to-event models in order to make stronger inferences regarding the direction of causation between the two phenotypes (Sellgren, Frisell, Lichtenstein, Landen, & Askling, 2014). The authors find that schizophrenia onset reduces subsequent onset of rheumatoid arthritis with a Hazard Ratio of 0.69, (95% CI = 0.59-0.80) consistent with our results from meta-analysis. This study has the considerable benefit of being performed on a population-level national registry population (n = 5,981,124), collected in Sweden between 1932 and 1989, and defining both schizophrenia and rheumatoid arthritis using hospital contacts. Thus there is strong evidence for a temporal relationship between schizophrenia and rheumatoid arthritis, both from our work and subsequent analyses.

Schizophrenia is the best-understood psychiatric disorder from a genetic perspective, with 128 genome-wide significant loci identified by GWAS; these alone explain 3.4% of

variance in schizophrenia risk on the liability scale, and a Polygenic Risk Score for schizophrenia explains 18.4% of variance in schizophrenia at the optimum threshold tested by the authors (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Therefore it is useful to consider the evidence for genetic factors responsible for the relationship between schizophrenia and rheumatoid arthritis. In our investigation of evidence for schizophrenia polygenic risk score (PRS) predicting rheumatoid arthritis case status, we use the PGC1 schizophrenia GWAS plus a subsample of Swedish genotypes (Ripke et al., 2013) as a base GWAS, and a target sample comprising rheumatoid arthritis cases from the WTCCC1 sample (Wellcome Trust Case Control Consortium, 2007) and controls from the RADIANT MDD study (Lewis et al., 2010), following rigorous quality control. This selection of controls is necessary, as the WTCCC1 controls are incorporated into almost every publically available GWAS data set, with the exception of PGC1-MDD. We find limited evidence for a significant relationship between schizophrenia and rheumatoid arthritis, with a nominally *positive* regression coefficient – which would indicate schizophrenia genetic risk increasing risk of rheumatoid arthritis, contrary to the epidemiological literature.

Since the publication of our study, there have been three relevant pieces of literature investigating the same question. The first (Stringer, Kahn, de Witte, Ophoff, & Derks, 2014) finds a strong effect of schizophrenia predicting RA, using very similar data to our study – the smaller and thus lower-powered PGC1 schizophrenia sample (Schizophrenia Psychiatric Genome-Wide Association Study, 2011) with the WTCCC1 controls removed as a base data set and WTCCC1 RA cases and controls as a target data set. The authors findings that schizophrenia PRS explains over 2% of variance in rheumatoid arthritis status, which is surprisingly large, as the proportion of variance in *schizophrenia* status explained by the PGC1 schizophrenia data in independent samples is, at most, 6-

7% at the most predictive threshold tested (Schizophrenia Psychiatric Genome-Wide Association Study Consortium, 2011). A possible concern is that sample overlap between base and target samples may not have been fully accounted for, leading to an over-estimation of the genetic overlap between these two traits. Alternatively, the results of this PRS analysis would indicate that the effects of environmental risk factors - such as the effect of medication - in the negative comorbidity between rheumatoid arthritis and schizophrenia would be even stronger than discussed in chapter 3, in order to mitigate the effect of genetic risk factors acting in the opposite direction. Thus our conclusions on the effects of environmental risk factors being responsible for the negative comorbidity between rheumatoid arthritis and schizophrenia observed in cohort studies is consistent with the results of Stringer et al.

Secondly, a study by Lee et al sought to validate a novel method to identify biological pathways responsible for phenotypes by investigating immune pathways related to schizophrenia (Lee et al., 2015). The authors build on the GREML method - which partitions heritability into the component explained by common genetic variation and error – in order to partition heritability into *a priori* defined biological pathways based on their associated genomic regions. This method bears similarity to MultiBLUP (Speed & Balding, 2014), which performs a similar calculation in a biologically agnostic framework, dividing the genome by physical distance. Lee et al test a set of genomic regions annotated to CD4+ memory T-cells based on an *a priori* model of the genetic aetiology of rheumatoid arthritis, yielding 87,651 SNPs; the authors find that genetic variation across coding, regulatory, DNase I hypersensitivity and intronic regions predicts heritability in schizophrenia and rheumatoid arthritis in inverse directions. The method developed in this paper is of great use in dissecting the biological pathways involved in disease, however the selection of appropriate candidate pathways without introducing

bias and appropriately correcting for the implicit number of multiple comparisons introduced by selecting a candidate pathway is challenging. It is unlikely that the stated effect size of the CD4 pathway on schizophrenia and rheumatoid arthritis could explain the strength of the epidemiological relationship seen, with a genetic correlation (r_g) of -0.046 (SE = 0.026) corresponding to a coheritability of -0.01 (P=0.036). Whilst this effect is non-zero, it is still not comparable to the epidemiological effect observed between these two disorders. Adding additional pathways to attempt to explain more of the epidemiological relationship between schizophrenia and rheumatoid arthritis would require increased multiple testing as more pathways with weaker priors would need to be added.

The third study of note validates our previous conclusions. Pouget et al perform a complementary analysis to our own, using GWAS data from a number of autoimmune disorders to predict schizophrenia within the PGC2-SCZ genotype data, using a polygenic risk score framework. The authors find a number of interesting relationships between autoimmune disorders and schizophrenia, validating several epidemiological relationships, such as a significant overlap between psoriasis and schizophrenia. When investigating schizophrenia and RA, however, the authors do not find evidence for a genetic overlap, consistent with our own findings. These results were presented as part of a conference symposium and are not currently available in print (Pouget, 2015).

The evidence for a genetic component to the epidemiological relationship between schizophrenia and rheumatoid arthritis is conflicted, with current literature finding a positive genetic correlation (Stringer et al., 2014), a negative genetic correlation in specific genomic regions (Lee et al., 2015) and no evidence for genetic correlation (Pouget 2015). In the context of this conflict, which will likely require further research to

disentangle, we did not find evidence in support of a genetic overlap. A number of explanations for this relationship have been proposed, and as non-genetic models they fall outside the scope of this thesis but will be discussed briefly. These include a protective effect of antipsychotic medication on rheumatoid arthritis. This theory is important, as anti-inflammatory medication such as tociluzimab – a TNF- α blocker – has already been trialled as an antipsychotic (B. J. Miller, Dias, Lemos, & Buckley, 2016). Evidence for the reverse – that anti-inflammatory medication may have an impact on psychiatric symptoms - is limited. There is evidence, however, that the burden of efficacy for an novel, repositioned, drug may be lower as it may have supra-additive effects when used within a combinatorial framework – for example, Choy et al find that aggressive combinatorial therapy is more effective than monotherapy in the treatment of rheumatoid arthritis (Choy et al., 2008). This suggests that the identification of novel pathways and drug targets within the autoimmune disorders may supplement and enhance existing therapies. Thus the value of verifying a non-genetic explanation for the relationship between pairs of disorders is still of great importance to the development of novel therapeutic strategies.

Depression

We investigate the epidemiological overlap between depression and autoimmune disorders in two very different cohorts. In a population-based cohort, the National Child Development Study, we use self-report data on depression to classify cases and controls, and investigate the prevalence of any autoimmune disorder – again based on self-report – amongst either group. We find an increased prevalence of any autoimmune disorder amongst depression cases, however individually low prevalences for each disorder impairs our ability for increased granularity. This is consistent with previous studies and

therefore provides good evidence for an epidemiological link between the two families of disorders that merits investigation into the direction of causality.

Our second investigation into the overlap between depression and the autoimmune disorders focuses on rheumatoid arthritis (RA) within the CARDERA study, a cohort of early RA patients. Rather than looking at the impact of case status alone on mental health, we use a number of metrics for RA symptom severity, and use a dimensional mental health scale – the MCS. This allows finer resolution into the impact of symptom severity of mental health and vice versa. We supplement findings on a population level by finding that increased RA symptom severity is associated with poorer mental health. In the case of both autoimmune disorder case status and autoimmune disorder symptom severity, we find evidence that these factors can increase risk of depression, but also that depression and low mood can increase risk of autoimmune case status, and worse prognosis and disease progression amongst RA cases.

Based on these findings, we propose considering comorbidity as driven by some shared risk factor as the most parsimonious solution. There is a wealth of literature arguing that a number of inflammatory markers are associated with Major Depressive Disorder patients relative to controls, including inflammatory cytokines (Maes, 1999; A. H. Miller, Maletic, & Raison, 2009). This parallels similar findings across many autoimmune disorders, including rheumatoid arthritis (Lubberts & van den Berg, 2001), Crohn's disease (Strober, Zhang, Kitani, Fuss, & Fichtner-Feigl, 2010) and Systemic Lupus Erythromitosis (Yap & Lai, 2013), amongst others. Furthermore, many other risk factors for depression are also associated with systemic inflammation, such as stressful life events (Danese et al., 2009; Danese et al., 2008; Dube et al., 2009).

Stressful life events are a well-established risk factor for Major Depressive Disorder. Literature on this link classifies stressful life events (SLEs) into dependant and independent, based on their cause. Dependent SLEs are thought to be driven partly by a patient's own behaviour, such as divorce or a change of job. Independent SLEs, by contrast, are defined by being caused by factors outside of a patient's control, such as being the victim of violent crime. This distinction is relevant when considering the evidence for a causal link between depression and the autoimmune disorders, almost all of which represent chronic and severe sources of impairment, negatively affecting factors such as quality of life, mobility and diet.

Mullins et al find that genetic risk of depression predicts number of dependent SLEs across depression cases and controls, but not independent SLEs (Mullins et al., 2016). Considering autoimmune disorders as a form of independent stressful life event - occurring independently of genetic risk of depression - aids the interpretation of our finding that depression genetic risk and autoimmune disorder status independently affect risk of depression in a population cohort.

Understanding the epidemiological relationships between phenotypes occurs at the level of the individual, whereas a study of the relationship between aetiological factors, such as inflammation levels and stressful life events, occurs at a molecular level. Here we term these to be more distal and more proximal respectively. Our findings above give merit to the investigation of an abnormal inflammatory profile as a causative agent in the epidemiological overlap between depression and the autoimmune disorders, however further work is required, investigating more proximal measures, in order to draw more definitive conclusions. Polygenic Risk Scores can be calculated using proximal phenotypes, such as individual differences in cell-surface receptor expression in the

immune system (Roederer et al., 2015), CRP levels (Dehghan et al., 2011) and personality traits (de Moor et al., 2012; Genetics of Personality Consortium et al., 2015; van den Berg et al., 2014); as data on these more proximal phenotypes becomes publically available, it will be possible to investigate evidence for a shared inflammatory profile between depression and autoimmune disorders at a population cohort level, and with autoimmune disorder symptom severity within clinical samples., where deeper phenotyping is possible

Leveraging Genetic Risk to Understand Aetiology, Prognosis and Treatment

Above we have presented the results of analyses that identify future research areas in the study of the psychiatric and autoimmune disorders. Of particular merit may be the antipsychotic effect of anti-inflammatory medication and the effect of systemic inflammation as a shared risk factor for a number of adverse outcomes later in life. Below we summarise the importance of using proximal phenotypes in the study of disease – in terms of aetiology, prognosis and treatment – and summarise ways in which genetic data can be used to determine a more useful proximal phenotype.

Proximal Phenotypes

A proximal phenotype, or endophenotype, biomarker, can be defined as a measurable biological trait, often requiring the use of a measuring instrument to detect, which is associated with disease risk without being defined itself as a disease. Examples of this would include urine glucose tolerance as a proximal phenotype for diabetes (Conn, 1940)

or a dimensional measure of neuroticism as a proximal phenotype for depression. We use the term proximal phenotype rather than endophenotype or biomarker, as the terms 'more proximal' and 'more distal' allow a greater appreciation for the dimensional nature of these traits and their relative importances, whereas biomarker and endophenotype imply that traits are either pathological or not - the reality is likely substantially more nuanced. This can be illustrated in the case of hypertension. Hypertension can be defined as resting systolic blood pressure above 140mm Hg, resting diastolic blood pressure above 90mm Hg, or both (Poulter, Prabhakaran, & Caulfield, 2015). This is a relatively arbitrary threshold, based on a large body of clinical evidence, however it may be inappropriate across different ethnicities and lifestyles. Hypertension is thus classified as a disease, not an endophenotype, however higher systolic blood pressure is associated in turn with a higher risk of chronic kidney disease (Krzesinski & Cohen, 2007) – i.e. it is an endophenotype or biomarker. Thus we believe it is more useful to describe this relationship as hypertension being a more proximal phenotype than chronic kidney disease, with systolic blood pressure being more proximal, biological factors affecting blood pressure - such as blood lipid levels - as yet more proximal, and the genetic factors affecting lipid levels as more proximal still.

More proximal phenotypes may be more useful than more distal phenotypes in the study of aetiology, prognosis and treatment of disease; regardless it is highly likely that they have been understudied compared to more distal phenotypes and thus merit at least equal consideration. Many of the most proximal phenotypes are continuous measures, providing increased resolution to make clinical judgements between patients. Secondly, a single more distal phenotype is likely to be influenced in a multifactorial way by multiple more proximal causes. This necessarily suggests that very similar clinical presentations may be differentiated by understanding their multifactorial causes. This in turn may

inform improved selection of treatment regimes – this is 'personalised medicine'. A substantial level of clinical heterogeneity has long been believed exist within multiple sclerosis (Lassmann, Bruck, & Lucchinetti, 2001), however only recently, incorporating genetic data, have these assertions been vindicated (Brynedal et al., 2007), and further study may inform the implementation of personalised treatment regimes.

Genetic Proximal Phenotypes

Under the biometric model (Galton, 1877), arguably all measurable biological traits have a multifactorial genetic origin – this is likely to be the case in particular for normally distributed traits as the central limit theorem suggests an aggregate effect across Bernoulli distributed genotypes. Individual alleles of small effect may thus be responsible for inter-individual variation in more proximal phenotypes – such as triglyceride level and neuroticism – as well as the intensively studied disease phenotypes, and we propose that understanding the genetic architecture of these more proximal traits would be of particular merit. The simplest way to achieve this would be to perform genome-wide association studies (GWAS) on each more proximal phenotype individually, however the diversity of more proximal phenotypes makes this prohibitively expensive and impractical. Below, we discuss ways to leverage genetic information and methodological novelty in order to construct better proxies for proximal phenotype-based risk of disease.

Pleiotropy – a scenario in which a given allele affects the expected values of more than one trait - is almost ubiquitous across human genetics. This is due to a number of factors including the way the concept of a trait is itself is defined. Medical disorders have traditionally been defined based on their phenomenology rather than their underlying aetiology – particularly as aetiology was often unknown at the time of definition. This

can be seen in the distinction between schizophrenia and bipolar disorder, which was first proposed by Kraepelin (Kraepelin, 1913); prior to this the two phenotypes were classified together. This dichotomy illustrates how a multifactorial phenotype is by definition a combination of proximal causes, and so overlap in risk factors - and thus pleiotropy and comorbidity - is inevitable between diseases. It is also possible that ubiquitous pleiotropy is a fundamental by product of evolution by natural selection; proteins perform multiple roles within an organism (Gould & Lewontin, 1979) and the catabolites – the downstream chemical products - of biological pathways frequently feed into multiple pathways in turn (Krebs, 1938; Krebs & Eggleston, 1938; Krebs, Salvin, & Johnson, 1938). Thus a number of genetic methods can exploit the pleiotropy between two disorders – something often manifested in comorbidity – in order to create a black-box metric for the aggregate risk conferred across more proximal but unmeasured – indeed unidentified – proximal phenotypes.

Polygenic Risk Scores as Proximal Phenotypes

As introduced above, the biometric model predicts that normally distributed traits will be influenced by many alleles of individually small effect. Some proportion of these can be identified at genome-wide significance by an adequately powered GWAS. Authors have previously found that a further number of risk-associated alleles may be included in a risk model by relaxing the genome-wide significance threshold from the typically used $\alpha = 5x10^{-8}$ to include variants with nominal association (International Schizophrenia Consortium et al., 2009). The weighted sum of thus identified risk alleles an individual carries is termed a Polygenic Risk Score (PRS), and is used extensively in this thesis as well as in the field of genetic epidemiology generally.

In discussing the idea of more proximal phenotypes, we consider that diseases are human constructs, representing the aggregate deviation from normality of one or several more proximal phenotypes. This assertion has direct relevance for GWAS and thus the construction of PRS and can be demonstrated by techniques such as pathway analysis, which cluster the significant regions from GWAS based on annotation to more proximal biological pathways. In the psychiatric disorders, where aetiology is still a source of active debate, this approach has proved a useful hypothesis-generating method – Breen et al (Network Pathway Analysis Subgroup of Psychiatric Genomics Consortium, 2015) cluster significant regions from schizophrenia, finding enrichment for variants involved in histone methylation, immune and neuronal/neurotrophic pathways and the synapse. Thus an appreciation that genetic risk of a more distal disease phenotype can be seen as an aggregate effect of polymorphism affecting a number of more proximal phenotypes informs the construction of more accurate polygenic risk scores.

Our first improvement to PRS (chapter 2) (Euesden, Lewis, & O'Reilly, 2015) indirectly exploits this fact. By optimising the computation of PRS through the development of the PRSice software program, we enable users to calculate the most predictive threshold at which to calculate PRS for a given pair of disorders, and for a given level of statistical power as determined by factors such as sample size and quality of phenotyping. We validate the increased predictive accuracy seen using PRSice, calculating PRS for depression cases and controls using GWAS data for schizophrenia, ever smoked cigarettes, and number of cigarettes per day. We find that schizophrenia genetic risk significantly predicts depression status, in line with previous findings (Cross-Disorder Group of the Psychiatric Genomics Consortium, 2013), and we report for the first time that genetic risk of ever smoking cigarettes also predicts depression status. This is

relevant to the notion of proximal phenotypes, as it suggests that some underlying biological pathways are influencing depression, tobacco use and schizophrenia. Furthermore, the exact proteins and genes involved in the pathway do not need to be known in order to perform useful prediction of phenotypes, and further downstream analyses such as stratification of cases. It is adequate and useful to consider the shared genetic risk between disease phenotypes as a means to calculate PRS and thus use this score as a proximal phenotype to measure risk of related traits.

The goal of these cross-trait methods is to exploit the existence of shared biological proximal phenotypes between disorders in order to predict genetic risk of a trait more accurately. Implicit in this is the idea that the shared genetic component between two genetically overlapping disorders will be at least nominally associated in a well powered GWAS of either, however will not necessarily contribute to the most significant associations in either GWAS. For this reason we develop a second method, PRSlice, which leverages the existence of a relatively small number of well powered GWASs for some traits, and a number of other disorders which overlap with these, in order to develop a novel biomarker for traits that have not yet been intensively studied by GWAS (chapter 6). By applying this approach to simulated data, where the genetic architecture can be reliably controlled, we find that PRSlice appears to outperform PRSice in scenarios where genetic correlation, r_G between base and target is modest but non-zero. We therefore propose that this may be used in scenarios predicting a target phenotype using a well-powered base GWAS on a different but correlated trait.

Future Directions

A number of future projects arise from work presented within this thesis. Within the study of schizophrenia and rheumatoid arthritis, the source of the epidemiological relationship has not yet been identified; verifying whether medication plays a role in this relationship will be important. Furthermore, it is possible that phenotypic and thus genetic heterogeneity within either RA or schizophrenia is masking a true effect of 'negative pleiotropy', and in this case deeper phenotyping on base (GWAS) and target samples will be instructive, as will incorporating methods explicitly designed to identify latent heterogeneity such as BUHMBOX (Han et al., 2016).

The hypothesis that inflammatory processes play a role in the overlap between depression and the autoimmune disorders may be elucidated further by using genetic risk scores that explicitly measure these proximal phenotypes. This is not possible in our analysis of the NCDS, as these individuals are included as controls in almost all publically available GWASs, with the notable exception of the PGC1-MDD GWAS, which we use here but is likely underpowered to identify any shared genetic component across depression and correlated phenotypes. We propose using other GWAS (base) phenotypes, such as CRP level – a proximal phenotype of inflammatory activity – in order to dissect the epidemiological relationship we observe further.

Alongside future directions in epidemiology, we also note the merit of investigating the effect of therapies for depression amongst RA patients. Cognitive Behavioural Therapy (CBT) has already been shown to improve the prognosis of RA patients over time (Evers, Kraaimaat, van Riel, & de Jong, 2002). Investigating CBT alongside

antidepressants, and their relative contribution to different components of poor mental health and different components of RA disease severity would be instructive in the management of this damaging and co-occurring phenotype. Furthermore, data on genetic risk may be incorporated in order to identify latent heterogeneity across patients.

Finally, there have been a range of novel methods investigating improvements to the calculation of genetic risk over the course of the last three years, and there are a range of future directions depending on the required scientific question and the available data. One such method we propose is an alternative to PRSlice, in which the genome is chunked into regions annotated to biological pathways rather than by physical distance. Genomic regions can be annotated to particular pathways, using publically available resources such as Gene Ontology (GO) and the Gene Set Enrichment Analysis (GSEA) Canonical Pathways library. The best threshold within each pathway is then determined separately, much like PRSlice. This allows us to leverage the considerable and growing understanding of the biological function of genomic regions in order to gain an insight into the biological causes of disease pathophysiology. Whereas PRSlice may be more sensitive to partially overlapping sets of risk variants between disorders showing some degree of genetic correlation, this method could be extended to explicitly prioritise biological pathways and thus drug targets for downstream trials. This method – Pathway PRS - is currently under development by our group (Ruan, Breen, & O'Reilly, 2015).

Conclusions

The pathway from identifying overlapping phenotypes with epidemiological methods to explaining the causes of this overlap by exploiting models of genetic risk forms the heart of this thesis. This is a relatively novel approach, partly due to the novelty of genetic risk models with adequate predictive accuracy. Applying these improved genetic risk scores to old epidemiological puzzles can add understanding to aetiology, and within a clinical setting add to understanding of prognosis and treatment. We have applied this paradigm to the overlap between the psychiatric and autoimmune disorders, finding little evidence for shared genetic components between schizophrenia and rheumatoid arthritis, and a body of supporting evidence for an inflammatory aetiology to depression, in line with other literature. Furthermore, we have developed two new methods to calculate more accurate markers for genetic risk, and proposed novel ways in which these might be applied in the future.

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