Depression, Inflammation, and Atopy: Examining a Complex Relationship Using Genetic Epidemiology

by

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Abstract

Depression is a common psychiatric disorder characterized by low mood, fatigue, concentration problems, and feelings of worthlessness. According to the World Health Organization, depression is the leading cause of disability worldwide. Depression is also associated with increased risk for a variety of medical conditions over the life course. While the exact etiologic mechanisms are unknown, depression is thought to involve interactions between a complex set of social, environmental, biological, and genetic risk factors.

The overarching theme of this dissertation is to use genetic methods to explore etiologic hypotheses about depression and its comorbidities. Genetic factors can influence depression risk, both directly and indirectly at multiple levels, including biological, behavioral, and the broader social and physical environment. The involvement of genetic influences in almost every layer of disease development makes genetic epidemiology a powerful tool for studying conditions such as depression that have complicated networks of contributing factors.

The first empirical chapter in the dissertation examines the relationship between atopic disorders (disorders involving inappropriate immune reactivity to benign stimuli, such as allergies and asthma) and psychiatric disorders, including depression. Although there is a known association between atopic disorders and depression, the relationship between atopic disorders and other psychiatric disorders is not as clearly established. Assessing this relationship is further complicated by the high levels of comorbidity between psychiatric disorders. Using data from a large, US-based sample, this chapter confirms the relationships between atopic disorders and a range of psychiatric disorders and determines that the relationships persist after adjustment for comorbid psychiatric disorders.

The second empirical chapter tests the hypothesis that there is a causal effect of interleukin-6 (IL-6) signaling on depression. IL-6 is a cytokine that plays numerous roles throughout the body, including pro-inflammatory signaling. Existing cross-sectional and longitudinal studies have report associations between elevated IL-6 and depression, and there are plausible biological mechanisms for how this cytokine may contribute to depression. Using data

from the United Kingdom (UK) Biobank, a large genotyped sample of UK adults, this chapter applies a Mendelian Randomization design to assess whether IL-6 signaling has a causal effect on depression. It finds evidence consistent with a causal effect of IL-6 signaling on depression, and that this relationship is likely to due to signaling via the soluble form of the IL-6 receptor.

The final empirical chapter applies multiple genetic analyses to explore competing explanatory models for the comorbidity between depression and atopic disorders, again within the UK Biobank sample. This chapter examines several potential explanations, including shared genetic liability, causal effects of atopic disorders on depression, differential self-reporting of atopy by individuals with depression, and lowering of the threshold for atopic responses among individuals with depression. From these analyses, this chapter shows that shared genetic influences on atopy and depression are likely, and that multiple explanations may contribute to the relationship simultaneously.

In sum, this dissertation illustrates that by using a variety of study designs from both traditional observational and genetic epidemiology, etiologic questions can be examined from multiple angles to produce a more robust understanding of complex relationships, such as the relationships between depression, inflammation, and atopy. This has resulted in several interesting findings, including that the relationship between atopic disorders and depression is at least partially explained by shared genetic liability, and that interleukin-6 inflammatory signaling is likely to have a causal effect on depression.

Chapter 1: Disentangling Complex Relationships Between Depression, Inflammation, and Atopy

Depression is a common mental disorder affecting approximately 7.3% of Americans each year[1] and is characterized by low mood, fatigue, concentration problems, and feelings of worthlessness.[2] It is associated with increased risk of physical comorbidities such as cardiovascular disease and type 2 diabetes.[1–3] According to the World Health Organization, depression is the leading cause of disability worldwide.[4] This underscores the need to clarify the etiologic processes and pathways that contribute to depression, which can inform the identification of modifiable factors to reduce risk of this condition.

While many elements of the etiology of depression are unknown, in general the development of psychopathology involves a complex set of social, environmental, biological, and genetic risk factors that interact over the life course. While the specific combinations of risk factors can vary between individuals, in general depression results from a combination of contributing factors rather than a single identifiable cause.[3,4] Genetic factors are estimated to explain approximately 37% of the variance in depression risk.[5] Studies of identical twins who differ in depression status illustrate the importance of social factors, such as current life stress and divorce, in predicting the onset of this condition.[6] Similarly, while onset of depression is often preceded by a stressful life event, genetic factors influence not only liability to developing depression following a stressor,[7] but also liability to experiencing life stressors that may precipitate a depressive episode such as serious illness or divorce.[8–11]

These examples illustrate that in addition to directly influencing disease risk, genetic factors can influence and interact with social and environmental risk factors. As a result, methodological approaches that leverage genetic epidemiology techniques can be a powerful tool for examining complex conditions like depression. To that end, the purpose of this dissertation is to apply genetic epidemiologic study designs and analytic methods to examine hypotheses about the etiology of depression and its relationship with medical conditions involving inflammation. Chapter 1 will use data from the Collaborative Psychiatric Epidemiologic Surveys (CPES), a set

of nationally-representative US surveys, to explore and quantify the cross-sectional relationships between atopic disorders (i.e., asthma and allergies) and several psychiatric disorders, including depression. Chapters 2 and 3 both use data from the United Kingdom (UK) Biobank, a large genotyped cohort of adults in the United Kingdom, as well as genome-wide association study (GWAS) coefficients from existing published studies. Chapter 2 will apply Mendelian Randomization, an approach used for causal inference, to assess the possibility of a causal relationship between inflammatory signaling and risk of depression. Chapter 3 will expand on the lessons learned from the first two chapters by applying multiple genetic study designs to assess potential explanations for the relationship between depression and atopy, including exploring competing explanatory models of this comorbidity.

This section provides an overview of the conceptual framework underlying the dissertation, and a brief summary of each chapter. Because the questions and methods in each aim are distinct, each chapter will also have a separate background section describing the rationale for that aim.

Biological, environmental, and social factors inter-relate to influence risk of depression

As discussed in Sapolsky (2017), human behaviors and experiences can be understood on a variety of levels simultaneously, with all involved levels being relevant to causality. A behavior or symptom caused by a particular neurobiological mechanism also has as contributing causes all environmental, psychosocial, genetic, evolutionary, and historical factors that led to the moment in which the neurobiological event occurred.[12] Although any phenomenon occurring in the brain (such as depression) has a proximal neurobiological cause, in most cases knowledge of the neurobiological mechanism is neither necessary nor sufficient to explain why the experience or behavior occurs. Examination of multiple levels of contributing factors provides a more complete explanation, as well as allowing for identification of levels at which intervention is most feasible.

Consistent with Bronfenbrenner's bioecological model,[13] risk factors for depression have been observed to operate on several different levels of causation, and to interact across levels. For example, many biological measures differ among individuals with depression when compared to individuals without depression, including alterations in cortisol responses to stress,[14,15] lower hippocampal volume,[16] and elevated levels of inflammatory biomarkers.[17] In

most cases it is not yet known whether these differences contribute to risk of depression, or whether they are caused by depression. There are also environmental exposures associated with depression, such as particulate matter air pollution,[18] pollen,[19] traffic noise,[20] and low neighborhood social cohesion.[21] These relationships may occur by way of biological mediators, such as inflammatory responses to air pollution and pollen.[22] Finally, numerous psychosocial factors also have relationships with depression, including childhood adversity, socioeconomic status, and social isolation. Psychosocial factors can influence factors at other levels. For example, socioeconomic status can influence exposure to several environmental risk factors,[23,24] and childhood adversity can impact cortisol regulation in adulthood.[25] Depression may also influence factors at multiple levels, for example a depressive episode may lead to weight gain, leading to both biochemical changes and increased exposure to weight-related social stigma.[26,27] Although it would be difficult for a single study to examine all levels of risk factors and their interactions simultaneously, understanding the complex interplay of factors that contribute to depression can help to ensure that individual studies of specific risk factors appropriately engage with the broader contexts in which they operate.

Inflammation as a potential etiologic mechanism for development of depression

Numerous studies have reported association between depression and elevated levels of inflammatory biomarkers.[28–32] Longitudinal studies report that elevated markers of inflammation (i.e., C-reactive protein (CRP), interleukin-6 (IL-6)) are associated with higher risk of developing depression,[28,32,33] and that depression is associated with subsequent elevations in inflammatory biomarkers.[33,34] There are several plausible biological mechanisms through which inflammation may play an etiologic role in depression, including the established phenomenon of inflammatory cytokines in triggering depression-like "sickness behavior" during illness or injury;[35] the effect of inflammation on synthesis of serotonin in the brain;[36] and reduction of hippocampal neurogenesis.[37,38] However, non-causal explanations are also possible: shared risk factors such as low socioeconomic status may contribute to both depression and inflammation,[39] and depression may lead to changes in immune regulation[40] that result in higher levels of inflammatory signaling.[41] Depression is also associated with health behaviors that can contribute to inflammation, such as poor diet or lack of exercise.[34] If the relationship between depression and

inflammation is causal, this would suggest anti-inflammatory medications may be a potential treatment for depression.[42] However, further study is needed to clarify the etiologic salience of inflammation for depression, and to determine which specific inflammatory signaling pathways are most directly relevant to the relationship.

Several lines of evidence support the existence of a causal effect of inflammation on depression. Individuals who receive the inflammation-stimulating drug interferon-alpha (used to treat hepatitis and some forms of cancer) are significantly more likely to experience depression as a side effect than individuals receiving other treatments.[43–45] Similarly, injection with the inflammation-provoking agent lipopolysaccharide (LPS) has been shown to produce depression-like behaviors in animal studies.[46] It is possible that inflammation-related depression may represent a distinct subtype, an idea supported by reports that elevated levels of inflammatory biomarkers are associated with lower probability of response to antidepressants,[47] and higher probability of response to treatment with anti-inflammatory medication (i.e., individuals with higher levels of tumor necrosis factor alpha (TNF-α) were more likely to have their depressive symptoms improve in response to infliximab).[48]

Clarifying the relationship is complicated by the fact that the nature of the relationship between depression and inflammation may vary across specific biomarkers due to the different roles each substance plays in the body. The most widely studied of these biomarkers is CRP, a protein produced by the liver as part of the acute phase inflammatory response.[49] Although the association between CRP and depression is widely replicated, causal inference study designs such as Mendelian Randomization do not support a causal relationship.[50,51] Other inflammatory biomarkers reported to be associated with depression include the cytokines interleukin-1β (IL-1β), IL-6 and TNF-α. All three of these cytokines have known mechanisms for interaction with the brain,[52,53] and multiple small studies have produced suggestive evidence that medications which inhibit IL-6 or TNF-α signaling may improve depressive symptoms.[42] Additionally, animal studies have also reported that blockade of IL-1β,[54] IL-6,[55] or TNF-α[56] signaling reduces depression-like symptoms in animals subjected to chronic stress.

The relationship between atopy and depression may involve inflammation

Atopic disorders are disorders in which an immune response is triggered by a benign environmental stimulus such as pollen or animal dander.[57] Common atopic disorders include

asthma, allergic rhinitis, and eczema. Asthma has been associated with increased levels of several serum inflammatory biomarkers, however not all asthma cases have an atopic etiology, and different asthma subtypes may have different inflammatory profiles.[58,59] The relationship between allergic rhinitis and systemic inflammation is more complex. While some systemic inflammatory effects have been reported,[60,61] other studies have reported decreases in levels of some inflammation-associated cytokines in response to allergen exposure,[62] possibly as part of a regulatory response to increases in levels of other cytokines.[63] Several differences in cytokine levels and other biomarkers of systemic inflammation have been identified in individuals with eczema, and may in part relate to genetic variants that alter skin barrier permeability, allowing higher exposure to allergens.[64,65]

Existing literature reports associations between atopy and several psychiatric disorders, including depression, anxiety, phobias, and panic disorder.[66–69] A recent meta-analysis of 51 studies reported that having an atopic disorder was associated with 1.59 times higher relative risk (RR) of depression (95% CI: 1.48-1.71), with similar odds ratios for asthma (RR: 1.59 95% CI: 1.46-1.74) and allergic rhinitis (RR: 1.57 95% CI: 1.27-1.93).[70] The high level of comorbidity between psychiatric disorders makes it difficult to distinguish whether each disorder has an association with atopy, or whether a relationship between one highly-prevalent psychiatric disorder and atopy may confound relationships between other disorders and atopy. In addition, although inflammation is one potential explanation for the relationship between depression and atopic disorders, other explanations are also possible. Symptoms of atopic disorders such as large rashes or asthma attacks may cause psychological distress, as can measures used to manage atopic disorders such as avoidance of pet ownership.[71,72] It is also possible that biological responses to depression-related stress and distress may lead to exacerbation of atopic responses,[73,74] or that individuals experiencing depression may be prone to somatization or to biased self-reporting of atopic status.[75,76] Additionally, other factors that influence inflammatory responses such as obesity[77] and inflammation-related genetic variants[78,79] may increase susceptibility to or severity of atopic disorders.

Genetic epidemiology offers opportunities to disentangle complex relationships

As illustrated by this brief review, disentangling complex networks of risk factors to determine the relationship between each risk factor and depression is a challenging task. This is particularly true in situations where several competing causal and non-causal explanations for a relationship are possible. An exposure such as elevated inflammatory signaling or history of atopic disorder may contribute to development of depression, as many longitudinal studies have suggested. However, this comorbidity may also be explained by a social or environmental factor affecting both traits such as socioeconomic status. It may also be explained by shared genetic influences affecting biological pathways common to both traits (co-heritability). And finally, it may be due to reverse causation, in which depression increases either the likelihood of experiencing the exposure or likelihood of reporting such an experience. Clarifying etiologic relationships requires the use of study designs and methods capable of distinguishing between these competing explanations.

Study designs using genetic data offer a unique means of evaluating the etiologic salience of competing explanatory models using observational data. Genes have a direct effect on many biological processes, and therefore have the potential to influence health outcomes, creating a clear role for genetic epidemiology in studying biologically-oriented questions (i.e., the role of inflammation in the development of depression). Genetic factors can also have a much wider-ranging effect, such as influencing how likely an individual is to experience a vast range of exposures, including smoking,[80] consumption of green vegetables,[81] and experiencing discrimination based on physical traits.[82,83] Genetic factors also act as effect modifiers of a variety of relationships, including influencing responses to exposures ranging from stress[84] to carcinogen exposure,[85] influencing progression from at-risk states to disease states (e.g., pre-diabetes to diabetes),[86–88] and influencing the severity and course of disease outcomes.[89–91]

Figure 1.1: Conceptual diagram

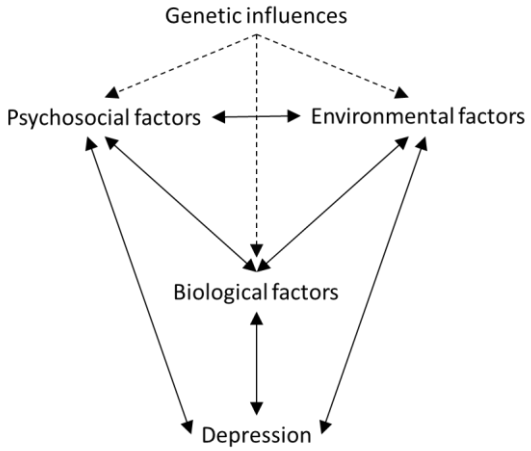


Figure 1.1 illustrates how genetic influences interrelate with psychosocial, environmental, and biological risk factors for depression

The involvement of genetic influences in almost every layer of disease development makes genetic epidemiology a powerful tool for

studying conditions with complicated networks of social, environmental, and biological risk factors such as depression. As shown in Figure 1.1, genetic factors (dashed lines) can influence or interact with many of the social and biological factors associated with depression, allowing for the use of genetic epidemiologic methods to examine a broad range of scientific questions. Genetic epidemiologic studies have the potential to provide useful information across levels. As one example, a study using genetic data may lead to improved understanding of inflammatory pathways relevant to depression, which could then support antidepressant drug development targeting the identified pathways, as well as supporting interventions targeting environmental and psychosocial risk factors likely to contribute to inflammation and depression.

Since the completion of the Human Genome Project nearly two decades ago, the genome-wide association study (GWAS), in which millions of genetic variants are each tested for association with a trait or disease, has become one of the most widely used analytic approaches in genetic epidemiology.[92] GWAS results can provide information about specific genetic variants associated with increased disease risk, and examining functional annotations associated with GWAS findings can help to identify biological pathways relevant to disease etiology.[93,94] Study designs using family-based data or genomic data can be used to estimate disease heritability, which describes the proportion of the variance in disease status that is explained by genetic influences.[95] Similar study designs can also be used to estimate co-heritability (i.e., the proportion of the covariance between two diseases that is explained by covariance between their genetic influences) and genetic correlation (i.e., a measure of how similar the genetic influences on two diseases are, independent of heritability). GWAS and other genetic epidemiology studies have helped identify common variants related to risk of several diseases including schizophrenia,[96] type 2 diabetes,[97] and several types of cancer.[98]

In addition to identifying genetic liability for disease, genetic data can also be leveraged to generate causal inferences about environmental risk factors. Relationships between exposures and outcomes, such as the relationship between inflammation and depression, can often be difficult to examine using observational data due to confounding by factors such as health behaviors and socioeconomic status.[99] Mendelian Randomization is a study design in which a genetic variant affecting an exposure (such as levels of a particular inflammatory marker) can be used to examine the relationship between that exposure and a health outcome such as depression. This design leverages the fact that genotype is a fixed trait established prior to birth and, subject

to certain assumptions, is distributed at random in the population. As a result, Mendelian Randomization provides a means to examine the relationship between inflammation and depression isolated from the influence of confounders and without the possibility of reverse causation.[100,101] This and other genetically-informed study designs will be applied throughout the dissertation to address several questions relevant to the relationship between depression, inflammation, and atopy.

Chapter 2: Examining the comorbidity between psychiatric disorders and atopic disorders

Chapter 2 examines the relationship between atopy and psychiatric disorders using data from the Collaborative Psychiatric Epidemiology Surveys (CPES), a set of large nationally-representative surveys based in the United States.[102,103] Many prior studies have reported associations between specific pairings of atopic and psychiatric disorders, such as the association between depression and seasonal allergies.[70] However, few existing studies have examined the relationship between multiple psychiatric and atopic disorders within the same cohort. This is important because comorbidity between psychiatric disorders is the rule, rather than the exception,[104] which means that any one psychiatric disorder may act as a confounder of the relationship between another psychiatric disorder and atopy (e.g., anxiety may confound the relationship between seasonal allergies and depression). Therefore, in order to clarify the specificity of the relationship between depression and atopic disorders it is necessary to account for psychiatric comorbidity in a rigorous manner. To address this concern, Chapter 2 examines the relationship between four of the most common psychiatric disorders (Major Depressive Disorder, Generalized Anxiety Disorder, Panic Disorder, and Post-Traumatic Stress Disorder) and two atopic disorders (seasonal allergies and asthma) while accounting for potential confounding by psychiatric comorbidity.

Chapter 3: Using Mendelian Randomization to evaluate whether the relationship between IL-6 signaling and depression is causal

Interleukin-6 (IL-6) is a cytokine that plays several roles within the body, including pro-inflammatory signaling.[105] Numerous cross-sectional studies have reported associations between

depression and IL-6, however it is not yet established whether these associations reflect a causal, as opposed to simply correlational, relationship. Results from animal studies, and the known role of IL-6 in producing depression-like "sickness behavior" during illness, suggest that a causal relationship is plausible. However, it is also possible that the association results from confounding by factors such as socioeconomic status and heath behaviors.[99] In addition, it is possible that the association between depression and IL-6 results from reverse causality in which biological or behavioral consequences of depression contribute to increases in inflammatory signaling.[34,106] To address these challenges, Chapter 3 uses data from the UK Biobank, a large cohort based in the United Kingdom, and several other studies to perform Mendelian Randomizations to assess whether the relationship between IL-6 signaling and depression is causal.

Chapter 4: Using genetic methods to examine the nature of the depression/atopy comorbidity

Chapter 4 builds on Chapters 2 and 3 by examining the nature of the relationships between depression and atopic disorders using genetically-informed approaches. There are several potential explanations for why depression co-occurs with atopic conditions, including non-causal explanations such as confounding by social and environmental factors or shared genetic influences that increase liability to both depression and atopy. In addition, there are bi-directional causal explanations such as the hypothesis that atopic disorders increase risk of depression by way of inflammation or another mechanism, or that immunoregulatory changes related to depression increase susceptibility to atopic reactions. Finally, it is possible that the observed association is largely driven by other factors such as differential self-reporting of atopic symptoms by individuals with depression, or by immune-related effects of depression altering the threshold for liability to atopic responses. To address the likelihood of each of these explanatory models of the comorbidity between depression and atopy, Chapter 4 uses cross-trait linkage disequilibrium (LD) Score Regression to examine shared genetic influences on depression and atopy, applies a Mendelian Randomization design to examine the possibility of a causal effect of atopy on depression, and uses polygenic risk score comparison to examine other hypotheses such as differential self-reporting and altered liability thresholds.

Conclusions

The relationships between depression, inflammation, and atopy are complex and multi-faceted. Disentangling this complex web of potential causal and non-causal relationships requires careful application of methods capable of distinguishing between competing causal explanations. Genetically-informed study designs offer unique opportunities to explore questions relating to disease etiology and biology. Better understanding these complex relationships will enable the creation of better strategies for identifying those at high risk, improving treatment and developing strategies for prevention of depression and its comorbidities.

Chapter 2: Allergies, Asthma, and Psychopathology in a Nationally-Representative US Sample[1]

Introduction

The term atopy refers to a propensity to develop an immunologic sensitivity to benign antigens typically tolerated by non-atopic individuals.[57] Atopic disorders are a family of disorders including allergic rhinitis, asthma, and some forms of eczema. Atopic disorders are highly heritable[107,108] and highly comorbid with each other,[109,110] suggesting they may share or have overlapping genetic susceptibility.[107,111,112] In susceptible individuals, exposures that trigger an atopic response may result in elevated levels of inflammatory biomarkers such as interleukin-6 (IL-6) and C-reactive protein (CRP).[113–117] Elevated levels of inflammatory markers have been associated with a range of mental health outcomes.[17,29,30,118]

Several studies have also suggested a relationship between common psychiatric disorders and atopic conditions. Large community-based studies have shown that major depressive disorder (MDD) is associated with both allergies[71,119–121] and asthma.[66,122,123] A link between panic disorder (PD) and asthma has been widely replicated,[123,124] and this relationship appears to be bidirectional;[125] only a handful of studies have examined the link between PD and allergies, but these also suggest a positive association.[126,127] Few studies have examined the relationship between generalized anxiety disorder (GAD) and atopic disorders, and results are mixed.[123,126,128,129] Finally, post-traumatic stress disorder (PTSD) has been associated with history of asthma,[123,130,131] however it is likely that this relationship is at least partially explained by trauma associated with asthma attacks.[132] There is little known about the relationship between PTSD and seasonal allergies, although one paper has reported an association.[133] Finally, experimental studies in animals have demonstrated that allergen exposure in sensitized animals can lead to increases in anxiety-like behavior and reductions in social behavior.[134]

[1] This chapter has previously been published using the same title in the *Journal of Affective Disorders*, May 2019, Volume 251, DOI: 10.1016/j.jad.2019.03.026

In sum, although multiple studies suggest a relationship between atopic disorders and common psychiatric disorders, the strength of this evidence varies across conditions. In some cases, evidence is limited to small, treatment-seeking clinical samples that may differ from the general population on factors such as disease severity and access to medical care.[135,136] Existing reports often examine only one atopic or psychiatric disorder in isolation, and thus it is unresolved whether these hypothesized relationships are consistent across a broad range of psychopathology or across multiple atopic conditions. Measurement of psychopathology also varies, limiting the ability to compare and evaluate disparate findings. Finally, because psychiatric conditions are highly comorbid with each other,[104] it is possible that if one psychiatric disorder were associated with atopy it could confound the relationship between other disorders and atopy, an issue that is rarely addressed in existing literature.

The objective of this study was to examine the relationships between two common atopic disorders, seasonal allergies and asthma, and several common psychiatric disorders using a large community-based sample. We hypothesized that both atopic disorders would show an association with a broad range of psychiatric disorders. We also hypothesized that earlier age of the onset for the atopic disorders would be more strongly associated with psychopathology than atopic disorders that onset later in life.

Methods

Sample

Data come from the Collaborative Psychiatric Epidemiology Surveys (CPES).[102] The CPES consists of three nationally-representative household surveys of US adults: the National Comorbidity Survey Replication (NCS-R), the National Latino and Asian American Study (NLAAS), and the National Survey of American Life (NSAL). All three studies were conducted from 2001 - 2003. All surveys of the CPES employed the Composite International Diagnostic Inventory (CIDI) to assess psychopathology as described below. Additional details of the CPES methodology are described elsewhere.[103]

The analytic sample for this paper is limited to CPES respondents who were asked about lifetime history of allergies or asthma (N=10,341 from the NLAAS and the long form of the

NCS-R only). The sample was further limited to respondents with complete data on the psychiatric disorders of interest (MDD, GAD, PD, PTSD), history of atopic disorders, and model covariates (N=10,309). Of the participants who were excluded for missing data, eight were missing data on lifetime history of allergies or asthma, and 24 were missing data on health insurance status.

For the analysis examining age-of-onset of atopic disorders in relation to psychopathology, the sample was restricted to respondents with a positive history of the atopic disorder in question and who provided valid data on their age at onset for the condition. Of the 3,512 individuals with a history of seasonal allergies, 3,290 (93.7%) provided a valid age at onset. Of the 1,202 individuals with a history of asthma, 1,137 (94.6%) provided a valid age at onset.

The institutional review boards at the University of Michigan, Harvard University, Cambridge Health Alliance, and the University of Washington approved the component surveys of the CPES.[137] This analysis used only de-identified publicly available data and was exempt from human subjects regulation.

Measures

History of psychopathology

Lifetime histories of MDD, GAD, PD, and PTSD were assessed using the DSM-IV version of the World Mental Health CIDI. This instrument consists of structured interviews based on the DSM-IV criteria for each disorder assessed,[138] and has been shown to perform well when compared to a clinician-administered semi-structured interview.[139] Respondents were considered to have a lifetime history of a disorder if they reported at least one period in their life during which they met the DSM-IV criteria for the disorder.[140] These disorders were selected because (a) they were assessed in both the NCS-R and NLAAS, (b) they were relatively common (had a lifetime prevalence >5%) (c) they had support from prior literature. Only a limited number of disorders could be selected due to the need to balance the exploratory nature of our research question with the analytic need to account for multiple comparisons.

History of atopic disorders

Lifetime histories of seasonal allergies and asthma were assessed by self-report. For allergies, respondents were asked "Have you ever had seasonal allergies like hay fever?". For asthma, respondents were asked "Did a doctor or other health professional ever tell you that you had asthma?" Although the accuracy of self-report assessment of atopic conditions like these may vary as a function of disease severity, previous studies of depression using clinician-verified asthma and blood measures have produced similar results to studies using self-reported measures [66,141–143]. Information about the age at onset of allergies and asthma was also assessed by self-report. Age of onset was categorized as childhood-onset (ages 0-12), teen-onset (ages 13-18), or adult-onset (age 19+). We chose to categorize this variable because our goal is to explore whether there is heterogeneity within the atopic disorder-mental health relationship as a function of when the atopic disorder developed;[144,145] as a result, modeling age at onset as a continuous variable would not be appropriate.

Other covariates

Age at time of interview (in years), gender, race, household income-to-poverty ratio (IPR), and health insurance coverage status were assessed by self-report. Initial covariate fit analyses showed that the relationship between age and psychopathology was non-linear. As a result, this variable was categorized as 18-29, 30-44, 45-64, and 65+. Race was categorized as "black", "Hispanic", "other", or "white" based on respondent self-report. IPR is the ratio of annual household income to the poverty threshold for the respondent's family size as determined by the US Census Bureau [146]. This variable was categorized into three levels: Income at or below the poverty limit, income two to three times the poverty limit, and income four or more times the poverty limit. Health insurance coverage was based on questions about the respondent's receipt of medical coverage through employer-provided health insurance, privately-purchased insurance, military medical coverage, Medicare, Medicaid, or other assistance programs or coverage, with respondents reporting having one or more of these classified as having health coverage, and individuals reporting none of these classified as not covered.

Analysis

Multivariable logistic regression models were used to assess the association between allergies and asthma and each of the four psychiatric outcomes. Each exposure was assessed in relation to four outcomes (MDD, PD, PTSD, and GAD); a Bonferroni-adjusted significance cutoff of 0.0125 was calculated to account for multiple testing.[147] Initial models were adjusted for age, gender, race, IPR and health insurance coverage. Following this, models were additionally adjusted for comorbid disorders (e.g., models of allergies predicting MDD were adjusted separately for PD, PTSD and GAD), to examine whether the relationships identified in the main model were confounded by psychiatric comorbidity. Controlling separately for each comorbidity increased the number of models from four to sixteen, however we retained our original Bonferroni-adjusted significance cutoff of 0.0125 because only four exposure/outcome associations were being tested. Finally, in instances where there was an association between an atopic and psychiatric disorder, logistic models were fit to examine whether the age-at-onset of the atopic disorder was associated with odds of the psychiatric disorder.

We then conducted a series of sensitivity analyses to assess the robustness of our findings. First, we expanded the outcome of panic from PD (approximately 4.1% of the sample had PD) to lifetime history of a panic attack (approximately 23.4% of the sample had experienced a panic attack), to assess whether the lack of association with asthma in the initial analysis was due to the small overall number of PD cases. Next, we examined whether the relationship between lifetime psychopathology and lifetime history of seasonal allergies replicated when using past-year history of each exposure and outcome; this analysis was conducted for allergies only, as history of asthma was only assessed over the lifetime, not in the past year. Finally, we examined whether including respondents from the NSAL in the analytic sample for asthma influenced our results; these respondents had been excluded from the main analysis because the NSAL did not include a question about seasonal allergies, but this survey did assess asthma and all the four psychiatric outcomes.

All analyses were conducted in SAS 9.4 using survey procedures to account for complex sample design of the CPES.

Results

Table 2.1 shows descriptive characteristics of the analytic sample by lifetime history of psychopathology. The columns are not mutually exclusive; respondents with more than one psychiatric disorder appear in multiple columns. As expected, psychiatric disorders were highly comorbid. For example, 24.1% of respondents with a history of MDD also had a history of GAD. Approximately one-third (36.6%) of the sample had a lifetime history of seasonal allergies, and 11.5% had a history of asthma. The atopic conditions were also highly comorbid, with 20.5% of respondents with seasonal allergies also reporting asthma, and 60.0% of respondents with asthma also reporting seasonal allergies.

Figure 2.1 shows the odds ratios and 95% confidence intervals of the relationships between seasonal allergies (green) and asthma (black) with each of the four psychiatric disorders. P-values are also included, to allow for comparison against the Bonferroni-adjusted multiple testing significance threshold of 0.0125. Seasonal allergies were significantly associated with MDD (Odds ratio (OR): 1.24; 95% Confidence Interval (CI): 1.06-1.46), GAD (OR: 1.54; 95% CI: 1.28-1.84), PD (OR: 1.53, 95% CI: 1.24 -1.91), and PTSD (OR: 1.32, 95% CI: 1.09-1.59). While all point estimates for asthma were greater than 1.0, asthma did not have statistically significant associations with any psychiatric outcome after adjustment for multiple comparisons. The sensitivity analysis examining the association between asthma and history of panic attack, instead of PD, also did not reach statistical significance (OR: 1.26, 95% CI: 0.93-1.70). As shown in Table 2.2 the relationships between allergies and each psychiatric disorder remained after controlling for psychiatric comorbidity. In some cases results were no longer significant after accounting for multiple comparisons, however effect estimates remained similar to those produced by the original models, indicating that adjustment for comorbid conditions did not attenuate the relationship.

We then examined whether age-of-onset (childhood, teen years, or adulthood) of atopy influenced the relationship between the disorder and psychopathology (Table 2.3). Among people with allergies, those whose allergies began before age 12 were 1.81 times more likely to have a lifetime history of PTSD than those whose allergies began in adulthood. There was no evidence that age of onset of allergies was related to the other three psychiatric conditions after adjustment for multiple comparisons.

Two sensitivity analyses confirmed the robustness of these results. As shown in Table 2.4, all associations between lifetime psychopathology and lifetime history of seasonal allergies

replicated when using past-year definitions of the exposure and outcomes. As shown in Table 2.6, the relationships between psychopathology and asthma still did not reach statistical significance even after including the NSAL in the analytic sample.

Discussion

This study used a large, community-based sample to simultaneously examine the relationship between multiple psychiatric disorders and seasonal allergies and asthma, two common atopic conditions. The primary finding of this study is that a history of seasonal allergies is consistently associated with greater odds of MDD, GAD, PD, and PTSD. Allergies that began earlier in life were more strongly related to likelihood of PTSD than those that began in adulthood. Contrary to expectation, there were no significant associations between asthma and any psychiatric disorder, although all effect estimates were in the expected direction. The results of this study indicate that the relationship between atopy and psychopathology may be more complex and nuanced than previously suggested.

The finding that seasonal allergies are associated with a broad range of psychopathology is consistent with prior literature. There is a widely-replicated association between MDD and allergies, and previous studies have suggested similar associations with allergies may exist for PD.[126,127] To our knowledge, this is the first study to report seasonal allergies as a possible risk factor for PTSD. Although trauma and adversity are risk factors for psychopathology in general, PTSD is unique in requiring a precipitating trauma as part of diagnostic criteria.[140] Informed by these results, future studies should explore whether atopy-related inflammation exacerbates psychiatric symptoms following trauma, or whether atopy-related systemic inflammation at the time of the trauma influences susceptibility to developing PTSD.

There are several potential mechanisms that may contribute to the observed relationship between allergies and this broad range of psychopathology. Systemic inflammation due to an atopic response may act on the brain in a manner that produces or exacerbates psychiatric symptoms.[17,118] In addition, inflammation from atopic responses during childhood or adolescence may influence development at critical periods,[148–150] consistent with the finding that earlier age of onset of allergies was more strongly associated with PTSD than allergies which onset later. Alternatively, genetic factors associated with propensity towards atopy may also be associated

with susceptibility to psychiatric disorders.[151,152] The finding that allergies are related to range of psychopathology may also suggest that allergies may be associated with an underlying factor common to several disorders, such as one of the domains, systems, or processes described in the National Institute for Mental Health's Research Domain Criteria (RDoC).[153,154]

Contrary to our expectations, asthma was not significantly associated with any form of psychopathology in our results. There are multiple potential explanations for these findings. First, many prior studies were conducted with child or adolescent samples,[155–157] and our analysis was restricted to adults. If the relationship between asthma and PD was stronger earlier rather than later in the life course, this may contribute to these disparate findings. However, the asthma-PD association has been replicated other large community-based studies of adults.[66,123,125] Another possible reason for the null results may be a result of how history of asthma was assessed, which was dependent on a reporting a physician diagnosis of this condition. This means that assessment of asthma could be correlated with access to medical care, a possibility we attempted to account for by adjusting for health insurance coverage status.

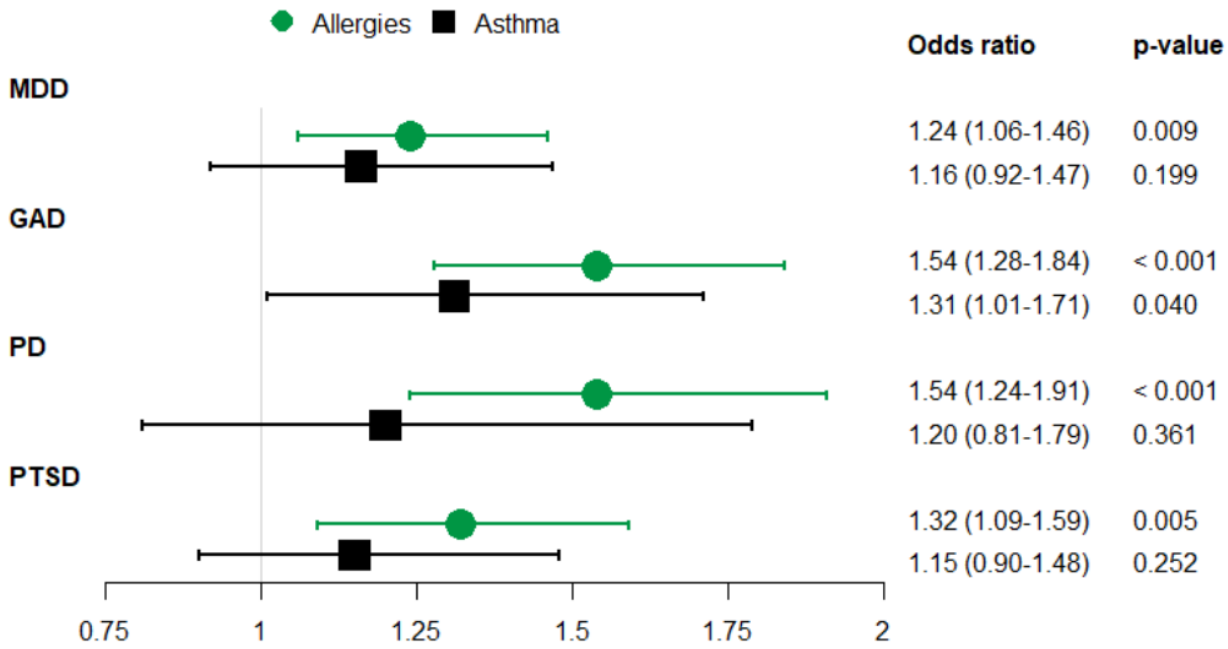
These findings should be interpreted in light of the study strengths and limitations. Data on history of allergies or asthma and their ages at onset was obtained via self-report. It is possible that individuals with a history of psychopathology may be more likely to report health conditions such as seasonal allergies, as has been found in another study of allergies and anxiety.[75] While we attempted to use age of onset information to address temporality, the retrospective design of this study (examining lifetime and past-year history) means that we cannot be certain of the directionality and temporality of the observed associations. However, because this design assesses occurrence of disorder in a longer period of time (one lifetime or one year) it avoids the possibility of confounding by seasonality, which would be a concern in a cross-sectional study due to the potential for seasonal patterns in atopic and psychiatric disorders. Finally, we do not adjust for medication use (either antidepressants or antihistamines) because we consider these drugs to be *proxy indicators* of our main exposures and outcome, so adjusting for these medications would not be appropriate for the analysis. This study also has several strengths, including the use of a well-validated diagnostic interview to assess a range of psychiatric disorders in a large community-based sample and multiple sensitivity analyses to assess robustness of the results. By examining multiple psychiatric outcomes assessed using the same instrument in the same sample, this analysis provides among the most complete assessments of

the relationship between allergies and asthma with common psychiatric disorders in a manner that allows for direct comparisons across these four conditions.

Further study is needed to understand the relationship between psychopathology and atopy. Longitudinal examination of underlying emotional, behavioral, and biological systems as they relate to atopy, consistent with the RDoC framework, are warranted.

Tables and Figures

Figure 2.1: Odds ratios for associations between atopic disorders and psychiatric disorders



All models adjusted for age, sex, race income-to-poverty ratio, and health coverage status. All results are given as OR (95% CI). The recommended p-value significance threshold is 0.0125, which applies a Bonferroni adjustment to account for the use of four tests per exposure.
MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.1: Descriptive characteristics of the main analytic sample

	MDD (n=2129)	GAD (n=935)	PD (n=577)	PTSD (n=771)	Whole sample (n=10309)
Age					
18-29	512 (21.9%)	163 (16.6%)	132 (19.7%)	181 (22.1%)	2608 (23.3%)
30-45	755 (33.6%)	321 (33.3%)	208 (37.2%)	266 (33.5%)	3519 (29.3%)
45-64	677 (35.5%)	355 (40.0%)	198 (35.1%)	283 (40.1%)	3082 (30.9%)
65+	185 (8.9%)	96 (10.1%)	39 (8.0%)	41 (4.3%)	1100 (16.6%)
Gender					
Male	708 (36.7%)	269 (32.7%)	173 (32.2%)	190 (25.4%)	4492 (47.3%)
Female	1421 (63.3%)	555 (67.3%)	404 (67.8%)	581 (74.6%)	5817 (52.7%)
Race					
Black	144 (7.2%)	70 (7.3%)	43 (7.0%)	85 (12.5%)	712 (11.1%)
Hispanic	536 (9.7%)	190 (6.6%)	140 (9.1%)	185 (8.4%)	3072 (11.7%)
Other	271 (5.4%)	91 (5.2%)	66 (6.7%)	73 (5.6%)	2356 (6.4%)
White	1178 (77.7%)	584 (80.9%)	328 (77.2%)	428 (73.6%)	4169 (70.8%)
Income-to-poverty ratio					
At or below poverty	510 (21.8%)	237 (21.8%)	165 (27.2%)	229 (26.3%)	2546 (22.0%)
2-3x poverty	561 (27.0%)	266 (28.7%)	175 (28.6%)	195 (25.6%)	2755 (27.8%)
4+ times poverty	1058 (51.3%)	432 (49.5%)	237 (44.2%)	347 (48.2%)	5008 (50.2%)
Has health insurance	1784 (85.6%)	802 (87.2%)	488 (87.1%)	645 (85.2%)	8518 (86.2%)
History of allergies	873 (43.2%)	429 (49.3%)	249 (49.2%)	339 (45.9%)	3503 (36.6%)
History of asthma	311 (13.4%)	142 (15.2%)	93 (14.4%)	129 (14.7%)	1195 (11.5%)
Allergy onset					
0-12	265 (32.6%)	126 (32.5%)	76 (33.3%)	120 (43.5%)	969 (32.6%)
13-18	125 (15.3%)	64 (16.0%)	48 (20.2%)	53 (15.8%)	543 (17.7%)
19+	423 (52.1%)	209 (51.6%)	110 (46.5%)	140 (40.6%)	1771 (49.7%)
Asthma onset					
0-12	116 (39.3%)	38 (31.2%)	22 (26.1%)	41 (34.9%)	510 (44.3%)
13-18	36 (11.3%)	22 (15.4%)	8 (11.0%)	17 (14.2%)	141 (11.9%)
19+	143 (49.4%)	68 (53.4%)	57 (62.8%)	61 (50.9%)	479 (43.8%)
Comorbid MDD	--	488 (52.2%)	232 (37.8%)	361 (43.7%)	--
Comorbid GAD	488 (24.1%)	--	171 (33.1%)	228 (29.6%)	--
Comorbid PD	232 (10.3%)	171 (19.6%)	--	144 (17.6%)	--
Comorbid PTSD	361 (17.4%)	228 (25.5%)	144 (25.7%)	--	--

Values are unweighted N, weighted percentages.
Individuals with multiple disorders appear in multiple columns. MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.2: Association between seasonal allergies and each psychiatric disorder, after controlling for each other psychiatric disorder

	MDD	GAD	PD	PTSD
Original model	1.24 (1.06-1.46)*	1.54 (1.28-1.84)*	1.54 (1.24-1.91)*	1.32 (1.09-1.59)*
Controlling for MDD	--	1.47 (1.24-1.74)*	1.49 (1.20-1.83)*	1.26 (1.05-1.51)
Controlling for GAD	1.16 (0.98-1.37)	--	1.41 (1.12-1.77)*	1.22 (1.01-1.49)
Controlling for PD	1.21 (1.03-1.43)	1.47 (1.22-1.77)*	--	1.27 (1.05-1.54)
Controlling for PTSD	1.21 (1.03-1.42)	1.49 (1.24-1.79)*	1.47 (1.19-1.81)*	--

* = Remains significant after adjustment for multiple comparisons.

All models are adjusted for age, sex, race, income-to-poverty ratio, and health coverage status. MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.3: Timing of atopic onset and risk of psychopathology

	MDD	GAD	PD	PTSD
Allergy age at onset (ref=19+)				
0-12	0.93 (0.73-1.20)	1.08 (0.79-1.48)	1.17 (0.84-1.61)	1.81 (1.28-2.55)*
13-18	0.81 (0.61-1.08)	1.01 (0.71-1.44)	1.42 (0.85-2.35)	1.27 (0.83-1.94)
Asthma age at onset (ref 19+)				
0-12	0.64 (0.45-0.92)	0.66 (0.34-1.31)	0.50 (0.23-1.12)	1.03 (0.53-2.01)
13-18	0.62 (0.31-1.25)	1.35 (0.58-3.11)	0.77 (0.28-2.10)	1.67 (0.80-3.50)

* = Remains significant after adjustment for multiple comparisons.

This model examines whether, given that an individual has an atopic disorder, a younger age-at-onset of allergies is associated with greater odds of psychopathology. Allergy N=3290, asthma N=1137.
Odds ratios are adjusted for age, sex, race, income:poverty ratio, and health coverage status.
MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.4: Association between past year seasonal allergies and past year psychiatric disorders in the main analytic sample

	Odds ratio
MDD	1.28 (1.04-1.57)
GAD	1.72 (1.38-2.14)*
PD	1.66 (1.22-2.26)*
PTSD	1.66 (1.28-2.15)*

* = Remains significant after adjustment for multiple comparisons.

All models adjusted for age, sex, race, income-to-poverty ratio, and health coverage.
All results are given as OR (95% CI).
MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.5: Characteristics of full CPES sample (includes NSAL) used in asthma sensitivity analysis

	MDD (n=2792)	GAD (n=1208)	PD (n=804)	PTSD (n=1179)	Whole sample (n=15428)
Age					
18-29	678 (21.8%)	219 (16.0%)	183 (18.2%)	304 (22.5%)	3942 (22.9%)
30-45	1002 (34.4%)	433 (35.1%)	289 (37.2%)	408 (34.0%)	5579 (29.9%)
45-64	885 (34.7%)	445 (39.2%)	281 (35.4%)	402 (38.8%)	4772 (31.1%)
65+	227 (9.0%)	111 (9.6%)	51 (9.2%)	65 (4.6%)	1825 (16.1%)
Gender					
Male	886 (36.8%)	346 (33.2%)	235 (33.3%)	368 (26.3%)	6683 (47.4%)
Female	1906 (63.2%)	862 (66.8%)	569 (66.7%)	911 (73.7%)	9435 (52.6%)
Race					
Black	627 (6.8%)	264 (6.4%)	210 (7.9%)	478 (14.8%)	5484 (10.8%)
Hispanic	556 (9.7%)	206 (6.9%)	148 (9.1%)	200 (9.0%)	3245 (11.8%)
Other	271 (5.1%)	91 (5.0%)	66 (6.7%)	73 (5.4%)	2356 (6.2%)
White	1338 (78.4%)	647 (81.8%)	380 (76.9%)	428 (70.9%)	5033 (72.2%)
Income-to-poverty ratio					
At or below poverty	758 (22.0%)	349 (22.5%)	269 (26.7%)	434 (27.3%)	4510 (21.6%)
2-3x poverty	808 (28.5%)	374 (29.7%)	244 (29.5%)	322 (25.9%)	4985 (29.2%)
4+ times poverty	1226 (49.4%)	485 (47.8%)	291 (43.9%)	423 (46.9%)	6623 (49.3%)
Has health insurance	2321 (85.4%)	1019 (85.6%)	673 (87.0%)	970 (84.4%)	13291 (86.4%)
History of asthma	434 (13.7%)	194 (14.9%)	135 (13.9%)	212 (14.9%)	1887 (11.5%)
Asthma onset					
0-12	116 (38.8%)	38 (27.1%)	22 (23.1%)	41 (29.9%)	511 (41.4%)
13-18	36 (11.3%)	22 (17.0%)	8 (12.6%)	17 (15.4%)	141 (13.1%)
19+	143 (49.9%)	68 (55.9%)	57 (64.3%)	61 (54.6%)	480 (45.5%)
Comorbid MDD	--	607 (53.3%)	306 (38.2%)	470 (44.1%)	--
Comorbid GAD	607 (23.9%)	--	213 (30.6%)	290 (29.5%)	--
Comorbid PD	306 (10.6%)	213 (19.0%)	--	201 (17.2%)	--
Comorbid PTSD	470 (16.6%)	290 (24.8%)	201 (23.4%)	--	--

Values are unweighted N, weighted percentages. Individuals with multiple disorders appear in multiple columns.
MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Table 2.6: Association between asthma and psychiatric disorders in the full CPES sample

	Odds ratio
MDD	1.19 (0.95-1.49)
GAD	1.29 (0.99-1.68)
PD	1.16 (0.80-1.69)
PTSD	1.21 (0.95-1.53)

All models adjusted for age, sex, race income-to-poverty ratio, and health coverage status. All results are given as OR (95% CI).
MDD = Major Depressive Disorder, GAD = Generalized Anxiety Disorder, PD = Panic Disorder, PTSD = Post-Traumatic Stress Disorder.

Chapter 3: Depression and Interleukin-6 Signaling: A Mendelian Randomization Study

Introduction

A large body of literature indicates that depression is associated with elevated levels of circulating inflammatory biomarkers.[17,53,118] The reasons for this association are not yet fully understood, and the association could operate by way of several different neurobiological pathways. The cytokine interleukin-6 (IL-6) has a widely-replicated association with depressive symptoms, and may represent a plausible biological pathway through which inflammation could lead to depressive symptoms.[29–32,158,159]

IL-6 can cross the blood-brain barrier, and circulating IL-6 interacts with the brain through the vagus nerve.[160,161] IL-6 is involved in brain signaling related to "sickness behavior", an adaptive response to illness or injury that leads to behavioral changes such as reduced appetite and decreased activity.[35,53,162] IL-6 signaling also leads to changes in tryptophan processing in the brain that result in reduced production of serotonin, and increased production of kynurenine and its neurotoxic byproduct quinolinic acid.[36,163] IL-6 signaling can also reduce neurogenesis in the hippocampus,[164,165] a reduction also observed in individuals with depression.[166,167] Experimental studies in humans and animals support the possibility of a causal relationship between IL-6 and depressive symptoms. A small human study (n=16) showed that injection of a low dose of IL-6 (0.5 μg/kg of body weight) produced short-term depression-like alterations in mood.[168] In mice exposed to experimental stressors, IL-6 receptor blockade[55] and IL-6 knockout mutations[169] have been found to reduce development of depression-like behaviors. Similarly, in rats, blocking IL-6 receptors reduced sickness behavior after injection with lipopolysaccharide, an inflammation-provoking agent.[162]

Like most cytokines, IL-6 interacts with cells via a receptor. The receptor for IL-6 exists in two forms, a membrane-bound form (IL-6R) used in classical IL-6 signaling and a soluble

form (sIL-6R) used in trans IL-6 signaling.[105] Classical IL-6 signaling occurs only in cells possessing a membrane-bound receptor (primarily immune cells and liver cells), where IL-6 has immunoregulatory, regenerative, and anti-inflammatory effects.[105,170,171] Trans IL-6 signaling occurs when IL-6 binds to sIL-6R in circulation, and the IL-6/sIL-6R complex is then capable of interaction with cells that have no membrane IL-6 receptors.[105,172] Most interaction between IL-6 and the brain occurs via the trans pathway,[173,174] and animal models have confirmed an important role for the trans pathway in neuroinflammation,[175] suggesting that IL-6 trans signaling via sIL-6R is more likely to be the relevant pathway in depression. However, mechanisms exist which could allow effects on the brain via classical IL-6 signaling, as shown in Figure 3.1. The use of drugs that inhibit IL-6 signaling has been suggested as a potential treatment for depression.[176,177]

Although it is plausible that IL-6 signaling plays a causal role in depression, alternative explanations for the association are also possible. One likely alternative is reverse causality, in which the symptoms and associated behaviors of depression lead to increased IL-6 signaling. Individuals experiencing depression are more likely to have poor health behaviors, including unhealthy diets, tobacco use, poor sleep habits, and reduced physical activity.[99,178] These health behaviors, in turn, are associated with increased inflammation.[179–181] Several of these behaviors are also associated with increased risk of obesity, particularly abdominal obesity, which is associated with increased inflammatory signaling due to the release of pro-inflammatory cytokines by adipose tissue.[182] Another possible explanation is confounding by a shared risk factor. Several known risk factors for depression are also associated with elevated inflammatory biomarkers, making them potential confounders of the depression-inflammation relationship. These include low socioeconomic status,[183] childhood adversity,[184] current life stress,[185] insufficient sleep,[186] and loneliness.[187]

Traditional approaches to analyzing population-based observational data do not differentiate between these competing explanations in a compelling way.[188] Mendelian Randomization uses a genetic variant with a known biological effect as an instrumental variable to assess the causal relationship between levels of a biomarker influenced by that genotype (the soluble IL-6 receptor) and an outcome (depression) independent of environmental confounding, as illustrated in Figure 3.2.[100] The Mendelian Randomization design is particularly useful in situations where there is the potential for a bidirectional relationship, because genotype is a fixed trait established before birth, and thus cannot be influenced by a health outcome (like depression)

developed later in life.[101] Mendelian Randomization is also useful in the management of confounding by environmental factors (e.g., socioeconomic status, stress) because a randomly distributed genotype is expected to be uncorrelated with these confounders. Given certain assumptions (random mating with respect to genotype and the absence of population stratification), genotype at the selected locus will be randomly distributed throughout the population, creating randomized "exposure groups" similar to those used in clinical trials.[188] The fact that genetic variants in or near the *IL6R* gene account for a large proportion (54.7%) of the variance in sIL-6R levels[189] makes it a suitable target for the Mendelian Randomization approach.

To our knowledge, one previous study has used Mendelian Randomization to report evidence consistent with a causal effect of IL-6 signaling on depression.[190] Further study is needed to confirm this relationship and to analyze the mechanisms through which it occurs. In this study we use Mendelian Randomization to test the hypothesis that IL-6 signaling has a causal relationship with depression. In additional exploratory analyses, we assess the robustness of the primary analysis and evaluate which IL-6 signaling pathway is involved in the relationship.

Methods

Study design

This study uses a two-sample Mendelian Randomization design, in which information about the relationship between the genetic variants and the exposure (circulating levels of sIL-6R) is obtained from an existing published genome wide association study (GWAS).[191] We then apply the regression coefficients and standard errors for the genotype-exposure variable relationship to the genotype and outcome (depression) data from the second sample with a similar ethnic background.[191] This two-sample approach makes it possible to examine a relationship between an exposure and an outcome even when a large sample measuring both characteristics in the same individuals is not available.[192]

Main analysis samples

We obtained coefficients for the genotype/sIL-6R association from two studies, to allow for replication of results across samples. The first study was van Dongen et al 2014[189], a GWAS of 4,846 Dutch participants that measured sIL-6R using an ELISA assay. We selected this study because the sample was unlikely to overlap with the UK Biobank sample, and because it included an additional GWAS conditional on the effects of rs2228145 (the SNP with the largest effect on sIL-6R). We also used the IMPROVE cohort GWAS[193] of 3,394 participants from multiple European countries which measured several proteins using an Olink array. We selected this study because the sample did not contain British participants and was therefore unlikely to overlap with the UK Biobank sample, and because full GWAS summary statistics were available.

We conducted GWAS to calculate coefficients for the genotype/depression association using data from the UK Biobank. We used two outcome phenotype definitions, "recurrent depressive symptoms" and "recurrent DSM-V major depression". To allow for replication across depression samples, we obtained additional coefficients for the genotype/depression association from the Psychiatric Genomics Consortium GWAS summary statistics for the Psychiatric Genomics Consortium 2018 meta-analysis of Major Depressive Disorder (PGC MDD 2018).[194] We selected this study because it used a large, well-phenotyped sample of European ancestry. The version of the summary statistics used in our analysis does not include data from 23andme, producing a final sample size of 59,851 cases and 113,514 controls.

We excluded individuals potentially included in PGC MDD 2018 (which included 29,740 individuals from a pilot release of UK Biobank genetic data) from our UK Biobank analysis. Although the UK Biobank data and PGC MDD 2018 data are never used together as part of the same Mendelian Randomization (a scenario under which sample overlap would create bias), we still chose to exclude sample overlap to ensure that replication of results across samples could not be driven by individuals common to both samples. The supplemental note contains additional information about the phenotype definitions and GWAS methods used with the UK Biobank data.

The samples and phenotypes used in the analysis are shown in Table 3.1, and additional details are provided in Table C.1 and Figures C.1-C.4.

Mendelian Randomization methods

We conducted Mendelian Randomization using several different methods: the Wald ratio of coefficients method,[195,196] the two-sample maximum likelihood method,[197] GSMR,[198] and PCA-IVW.[199] These methods differ in several important aspects including statistical power, requirements for instrumental SNP selection, and availability of diagnostic tests to check the Mendelian Randomization requirements, allowing the strengths and weaknesses of the selected methods to complement each-other. Consistency of results across multiple methods helps to confirm the robustness of the results and ensure that they do not result from biases particular to one Mendelian Randomization method.[200,201]

For the single-SNP analysis using the Wald ratio of coefficients method,[195,196] we selected the biallelic SNP rs2228145. Rs2228145 is a missense variant in a proteolytic cleavage site necessary for the release of IL-6R in its soluble form, which explains approximately 51% of the variance in sIL-6R levels, making it a strong instrumental variable for Mendelian Randomization.[189] In datasets where information for rs2228145 was not available, we used the SNPs rs4129267 and rs12126142 as proxies, because they have r^2 values greater than 0.99 with rs2228145 in UK Biobank and in the 1000 Genomes EUR population.[202] With the Wald ratio of coefficients method, the causal effect estimate is produced by dividing coefficient for the association between the instrumental SNP and the outcome ($\beta_{Y|Z}$) by the coefficient for the association between the instrumental SNP and the exposure ($\beta_{X|Z}$),[196] as shown in Figure 3.2 and Equation 3.1:

Equation 3.1: Wald Ratio of Coefficients

$$\hat{\beta}_{Y|X} = \frac{\hat{\beta}_{Y|Z}}{\hat{\beta}_{X|Z}}$$

We also used the two-sample maximum likelihood method,[197] which combines information from multiple independent SNPs to produce a causal effect estimate. We used multiple methods to select independent SNPs, which are discussed further in the supplemental note. In order to make sure the effects of rs2228145 could be easily examined in visual plots, we ensured selection of this SNP (or its best-available proxy) by excluding other SNPs in close LD with it prior to SNP selection. All analyses were performed in R 3.6.0 using the package

TwoSampleMR 0.5.4. We used additional diagnostics to ensure the quality and consistency of the results. These included MR-Egger regression to check for SNPs that had an association with the outcome through a mechanism other than the exposure,[189] Cochran's Q to test for heterogeneity in per-SNP estimates of the odds ratio, and leave-one-SNP-out analyses to confirm that no single SNP produced large changes in the estimated causal effect.

Mendelian Randomization analyses using selected independent SNPs are sensitive to the specific SNPs used in the analyses, particularly when only a small subset of all eligible SNPs can be selected.[199] To address this limitation, we used two methods that can account for LD, PCA-IVW[199] and GSMR,[198] to allow for inclusion of a greater number of SNPs and to improve the statistical power of the analysis. GSMR can account for moderate levels of LD, allowing for a more lenient LD clumping threshold, while PCA-IVW uses principal components and eliminates the need for LD-based SNP selection entirely. The GSMR analysis was performed using GCTA 1.92.2 beta, with r^2 clumping thresholds ranging from 0.05 to 0.2 (Table 3.2). During this analysis, we used the HEIDI-outlier test to exclude any SNPs detected to have an association with the outcome through a mechanism other than the exposure.[198] The PCA-IVW analysis was performed in R 3.6.0 using code from Appendix A of Burgess (2017).[199] For the PCA-IVW analysis, we included all SNPs having at least a suggestive association with the exposure ($p < 1 * 10^{-6}$) and obtained data for the SNP correlation matrix using the UK Biobank sample and GCTA 1.92.2 beta.

Exploring potential mechanisms underlying IL-6 signaling and depression

The strongest SNP in the main analysis, rs2228145, affects IL-6 signaling in two ways: the minor allele increases signaling via the trans pathway by increasing sIL-6R levels, but it also reduces signaling via the classical pathway (Figure 3.1).[203,204] It is possible that other SNPs used in the analysis may also be associated with both pathways because most sIL-6R GWAS results are near the *IL6R* gene and could be in partial LD with rs2228145. Therefore, we performed several exploratory analyses to examine which of the IL-6 signaling pathways might serve as the mechanism for a causal effect on depression and to assess the impact of LD with rs2228145.

Exploratory analysis: Samples

The exploratory analyses used samples from the main analysis and several additional studies. For soluble glycoprotein 130 (sgp130), we used coefficients from the KORA study[205] and the Framingham Heart Study.[206] For *IL6R* eQTLs, we used coefficients from GTEx v8,[207] CAGE,[208] and Westra 2013.[209] The Westra 2013 coefficients were provided as Z-scores, which we converted to betas and standard errors using formulas from Zhu 2016.[210] Finally, for CRP, we used coefficients from the KORA study.[205]

Exploratory analyses: Approach

First, we conducted an analysis using CRP as a proxy for classical IL-6 signaling (i.e. signaling via the membrane receptor, which stimulates CRP production).[204] We selected CRP as a proxy because for SNPs that are in or near the *IL6R* gene, any association between these SNPs and CRP is likely to result from their effects on classical IL-6 signaling. We selected SNPs from the IMPROVE sIL-6R GWAS results to obtain a set of SNPs that produced significant single-SNP causal effect estimates for the effect of sIL-6R on depression. We then used the KORA coefficients for the associations between these SNPs and CRP, and estimated the "apparent causal effect" of CRP on depression using the Wald ratio of coefficients method. This analysis is depicted in Figure 3.3 panel B.

Second, in addition to using r^2 to assess LD between each SNP and rs2228145, we also examined Lewontin's |D'| statistic[211] because |D'| is not as severely affected by differences in allele frequency and may detect LD in some cases where r^2 does not (illustrated in Figure C.5).[212] We then attempted to exclude the effects of rs2228145 by conducting additional Mendelian Randomization analyses using only SNPs having both $r^2 \leq 0.01$ and $|D'| \leq 0.15$ with rs2228145. This analysis is depicted in Figure 3.3 panel C.

Third, we conducted a Mendelian Randomization analysis examining soluble glycoprotein 130 (sgp130), a protein which inhibits IL-6 signaling only through the trans pathway (signaling via sIL-6R, as shown in Figure 3.1).[105] If the trans signaling pathway were the mechanism for the causal relationship, higher levels of sgp130 would be predicted to have a protective effect against depression. This analysis is depicted in Figure 3.3 panel D.

Fourth, we conducted an analysis of eQTLs for the *IL6R* gene using the three eQTL datasets shown in Table 3.1. In cases where a dataset included data for more than one expression probe for *IL6R*, we used the probe that had the largest number of significant eQTLs. We expected these eQTLs to increase levels of one or both forms of the receptor, potentially allowing for examination of the effect of increased receptor availability without an accompanying decrease in another pathway. This analysis is depicted in Figure 3.3 panel E.

Ethical approval

This analysis used only de-identified data (UK Biobank) and summary statistics (all other samples) and was therefore exempt from human subjects regulation.

Results

Table 3.1 provides details of the studies and samples used in the analyses. All study participants were at least 18 years old and of European ancestry and all studies included both males and females. All eligible significant SNPs for sIL-6R were located on chromosome 1.

In the main analysis (Table 3.2), across all combinations of samples and methods, the majority of associations were significant, indicating that higher levels of sIL-6R were associated with increased odds of depression. For example, using the PCA-IVW method with the van Dongen and UK Biobank samples, a 10^{-8} g/mL increase in sIL-6R was associated with 1.023 times higher odds of depression (95% Confidence Interval: 1.006 - 1.039, p=0.006). Furthermore, even analyses which did not reach significance produced odds ratios greater than 1.0 (consistent with the significant results). The consistency of the findings across the various combinations of exposure and outcome samples and across analytic methods indicates that the results are robust to differences in samples and analytic methods. We then repeated the main analysis using the "recurrent DSM-V major depression" phenotype, which is a more stringent definition but produces a smaller sample size because it can only be evaluated in participants who completed the UK Biobank Online Mental Health supplement. Despite this smaller sample, most analyses still produced significant or near-significant results (shown in Table C.4), and the direction of all odds ratios remained consistent and positive.

We conducted a series of analyses to explore whether the identified causal relationship between IL-6 signaling and depression is primarily a function of the classical or the trans signaling pathway. Table 3.3 shows the results of the analysis using CRP as a proxy for classical IL-6 signaling. The SNPs shown in Table 3.3 are drawn from the significant SNPs from the two sIL-6R GWAS used in Table 3.2. The results of Table 3.3 show that these SNPs may be associated with reduced activity of the classical IL-6 signaling pathway; this is in addition to their effects on the trans pathway shown in Table 3.2 and is thus consistent with pleiotropy. This analysis demonstrates that evidence could also be consistent with reduced signaling via the classical IL-6 pathway as the mechanism for the relationship between these SNPs and depression. The two SNPs at the bottom of Table 3.3 have little to no linkage disequilibrium (LD) with rs2228145 and are the only SNPs for which the odds ratios calculated using CRP do not match the direction of effect of rs2228145. The effect estimates produced by these SNPs are still consistent with a causal effect of higher sIL-6R on depression, but no longer consistent with a causal effect of decreased classical IL-6 signaling on depression, supporting signaling via the trans/sIL-6R pathway as the relevant mechanism for the causal relationship.

Table 3.4 extends the findings from Table 3.2 by repeating the Mendelian Randomization of sIL-6R with additional filtering to exclude the effects of rs2228145. Although the genetic instruments used in this exploratory analysis were not as strong as rs2228145, in several cases these analyses still suggested a relationship between IL-6 trans signaling and depression. In all filtered analyses producing significant or near-significant p-values, the effect estimates were above 1.0, illustrating that the relationship between sIL-6R and depression does not reverse direction when excluding the effect of rs2228145. We obtained similar results when using SNP coefficients from the van Dongen (2014) conditional analysis that estimated SNP coefficients for sIL-6R while adjusting for rs2228145 (shown in Table 3.2 as "PCA-IVW (conditional)"). Although the effect estimates from the analyses using the conditional GWAS coefficients were not statistically significant, the direction and magnitude of the estimated ORs was consistent with the other results in Table 3.2. However, using conditional GWAS coefficients may not fully account for LD with a SNP that has very strong effects,[213,214] consistent with the pattern shown in Figure C.14.

The Mendelian Randomization results for soluble glycoprotein 130 (sgp130) were not statistically significant (Table C.5). This null finding may indicate that the inhibitory effects of

sgp130 on IL-6 trans signaling are not protective against depression. However, it is also possible that the SNPs used in this analysis were not sufficiently strong genetic instruments. Additionally, the *IL6ST* gene that encodes sgp130 also encodes a membrane-bound form of gp130 that is used in both IL-6 signaling pathways, so some SNPs used in this analysis may have had pleiotropic effects.

Finally, the Mendelian Randomizations using *IL6R* eQTLs consistently produced effect estimates below 1.0, suggesting that genetically-controlled increases in expression of the *IL6R* gene is associated with lower risk of depression (Table C.6). These estimates should be interpreted with caution, however because all eQTLs were in at least partial LD with rs2228145, and the major allele of rs2228145 is associated with higher expression of the *IL6R* gene (as well as higher CRP and lower sIL-6R). This interpretation (i.e., confounding due to rs2228145) is further supported by the fact that the expression-increasing alleles of all *IL6R* eQTLs had positive coefficients for their association with CRP, as shown in Figures C.16-C.18.

Discussion

The results of the primary analysis are consistent with a causal effect of IL-6 signaling on risk of depression. This study used multiple Mendelian Randomization approaches to estimate this relationship, and the effect estimates were largely consistent regardless of the specific analytic approach used. These results build on existing cross-sectional[29–31] and longitudinal[28] studies suggesting a role for IL-6 signaling in depression. The results also strengthen evidence regarding the causal nature of the relationship between inflammation and depression by examining it in a manner that establishes both directionality and independence from environmental confounders. Although non-causal explanations this relationship are still possible, these results in combination with evidence from other Mendelian Randomization [215] and animal [55,162,169] studies support the theory that the observed relationship is at least in part causal.

Although the effect estimates were fairly small (i.e. odds ratios ranging from 1.008 to 1.045 in Table 3.2), it is likely that this reflects a variety of factors including the units used to measure the exposure in each analysis, the heterogeneity of depression and the possibility that inflammation may play a causal role in only a subset of cases,[216,217] and the possibility that the causal effect measured is not direct (eg. the odds ratios may reflect the effect of receptor

availability on the effect of interleukin-6 on depression). Observational studies measuring interleukin-6 itself often produce larger effect estimates,[218] so the true effect of interleukin-6 on depression may be considerably larger than the effect estimate produced when using its receptors for Mendelian Randomization.

The results of this study are consistent with either a causal effect of *increased* trans IL-6 signaling or of *decreased* classical IL-6 signaling as the mechanism underlying risk of depression. Given the existing literature showing that depression is generally associated with higher, not lower, levels of IL-6 [29–32,158,159], increased trans signaling appears more likely as a mechanism than decreased classical signaling. We conducted a series of exploratory analyses to determine which IL-6 signaling pathway was the driver of the causal effect. While the results of these exploratory analyses were not definitive, the preponderance of the evidence favors IL-6 trans signaling as the relevant pathway underlying this relationship. Finally, it is important to note that even if a causal effect could be isolated to one IL-6 signaling pathway, this would not necessarily indicate a *direct* causal effect (either of sIL-6R or IL-6 itself), because IL-6 signaling impacts several other biological pathways which may mediate its relationship with depression.

The nature of the relationship between depression and IL-6 signaling is of particular interest due to ongoing efforts to develop depression treatments which target IL-6 signaling. Tocilizumab, a drug currently undergoing trials as a potential depression treatment,[177,219] binds to both IL-6 receptor types and inhibits signaling via both pathways. The results of the tocilizumab trials may eventually provide the opportunity to distinguish which of the IL-6 signaling pathways is involved in the causal relationship. If the relationship between rs2228145 and depression occurs by way of increased trans IL-6 signaling, then tocilizumab's inhibition of trans signaling might be expected to succeed as a depression treatment. However, if the relationship between rs2228145 and depression occurs by way of decreased classical IL-6 signaling, tocilizumab's inhibition of classical signaling might be expected to exacerbate depressive symptoms. If the relationship between IL-6 signaling and depression is confirmed to occur via the trans pathway, drugs which affect only the trans pathway may also have potential as depression treatments.[220,221]

Strengths and limitations

The primary limitation of this study is that most of the SNP instrumental variables for sIL-6R levels are located on chromosome 1 near the *IL6R* gene, making it difficult to distinguish between effects specific to each SNP and effects stemming from SNPs being in partial LD with rs2228145. Although we conducted a series of exploratory analyses to attempt to remove the effect of rs2228145, including the use of conditional analysis coefficients and stringent LD filtering, we could fully not confirm that any analysis was fully independent of the effects of rs2228145. Although the pleiotropic effects of rs2228145 on both IL-6 signaling pathways make it difficult to make inferences specific to either signaling pathway, since both effects of this SNP are on IL-6 signaling pathways, it is still appropriate to conclude that results support a causal effect of IL-6 signaling on depression. Additionally, sample overlap is likely between the van Dongen (2014) coefficients and the PGC MDD 2018 coefficients because both studies include individuals drawn from the Netherlands Twin Register (NTR) cohort; however, we used multiple combinations of exposure and outcome samples to ensure that results were robust to overlap occurring with any specific pair of samples. Finally, this analysis was limited to participants of European ancestry and future research should extend this work to more diverse samples.

This study also has several strengths, including the use of large well-characterized samples (e.g., UK Biobank and PGC), the analysis of multiple proteins and measures related to IL-6 signaling pathways, and the consistency of results across multiple Mendelian Randomization approaches and samples. As discussed in Lawlor (2016), using multiple methods to "triangulate" inquiry into a research question is an effective means of approaching complex questions, such as those involved in attempting to clarify suspected causal relationships.[200] These findings help clarify the role of inflammation in the development of depression, and suggest several pathways for future research that can inform efforts to both prevent and treat depression.

Conclusions

The findings from this study are consistent with a causal effect of IL-6 signaling on depression. Although we were not able to definitively isolate which of the two IL-6 signaling pathways is the mechanism for the causal effect, our exploratory analyses in combination with evidence from other studies provides some support for the trans signaling pathway as the probable mechanism. These results encourage exploration of therapies that influence IL-6

signaling as possible treatments for depression. Future research should also investigate the role of IL-6 signaling as a mediator of the established associations between depression and modifiable risk factors such as poor diet,[99] chronic stress,[185] and physical inactivity.[181]

Tables and Figures

Figure 3.1: Interleukin-6 signaling pathways

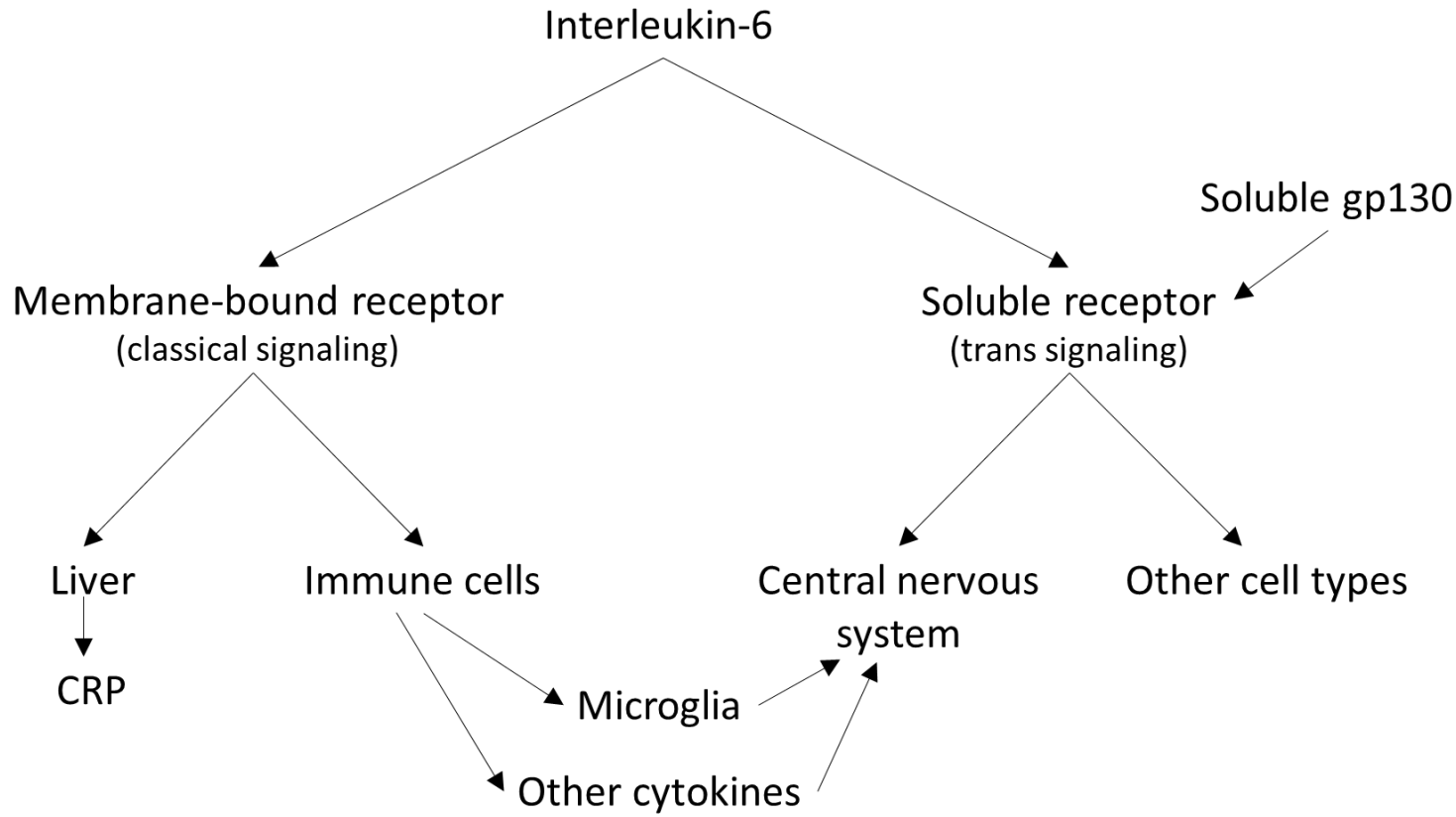


Figure 3.1 illustrates the two IL-6 signaling pathways. The trans signaling pathway is regarded as the more plausible mechanism for a relationship between IL-6 and depression due to the important role of trans signaling in the central nervous system. However, mechanisms exist through which classical signaling could influence the central nervous system, including the role of classical IL-6 signaling on immune regulation (which may influence other inflammatory signaling chemicals that then interact with the brain) and the presence of membrane IL-6 receptors on some microglia. (gp130 = glycoprotein130, CRP = C-reactive protein, IL-6 = interleukin-6).

Figure 3.2: The Mendelian Randomization study design

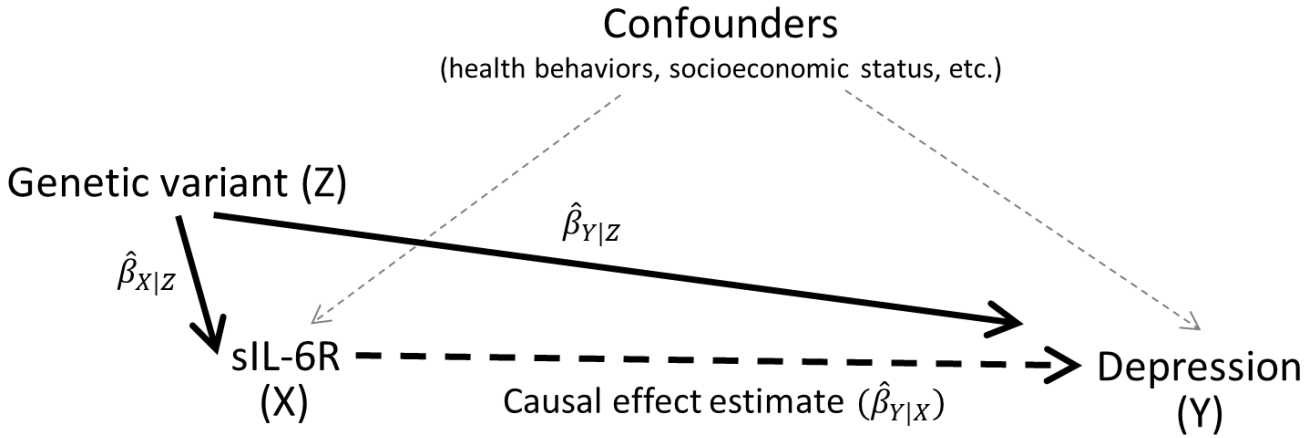


Figure 3.2 illustrates the Mendelian Randomization study design. The relationship between a genetic variant and the exposure ($\hat{\beta}_{X|Z}$) and the relationship between a genetic variant and the outcome ($\hat{\beta}_{Y|Z}$) are measured and used to estimate the causal effect of the exposure on the outcome ($\hat{\beta}_{Y|X}$) independent of confounders.

Figure 3.3: Visual overview of analyses and their relationships with signaling pathways

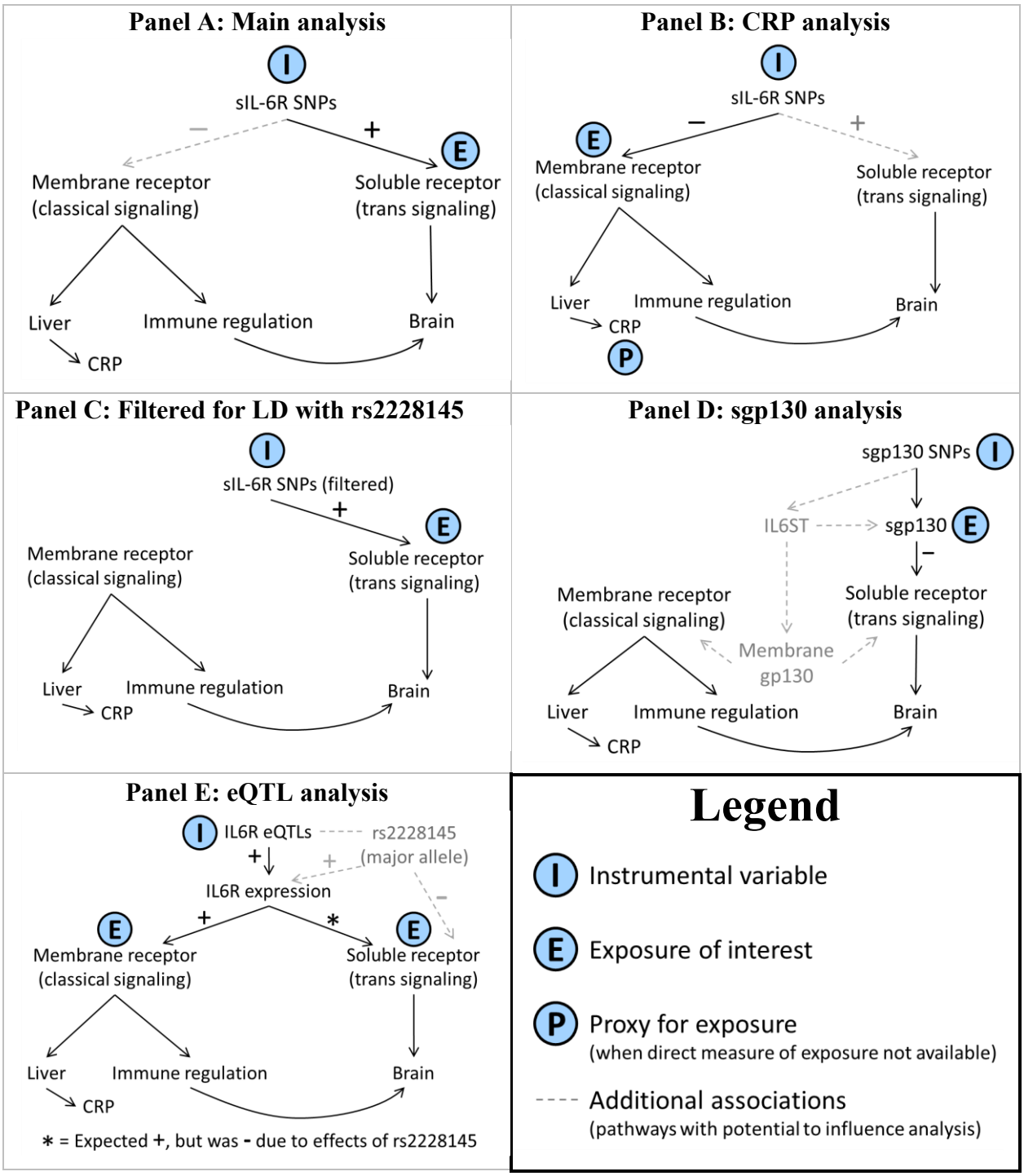


Figure 3.3 illustrates the analyses used in this chapter, and how each analysis relates to the classical and trans signaling pathways. The main analysis (A) and CRP analysis (B) both use SNPs significantly associated with sIL-6R levels, demonstrating that LD with rs2228145 makes it possible for results produced using these SNPs to have occurred via either pathway. Panels C-E illustrate analyses that attempted to isolate the causal effect to one of the two signaling pathways, and (in D and E) also illustrate additional relationships that could interfere with the analysis' ability to isolate the causal effect to one pathway.

Table 3.1: Samples and summary statistics used in the analysis

Sample	Phenotype	N	Age range	Gender composition	Format
van Dongen 2014[189]	sIL-6R blood levels	4,846	18-90	61.3% female	GWAS coefficients
IMPROVE[193]	sIL-6R blood levels	3,394	55-79	Includes males and females	GWAS coefficients
UK Biobank[222]	Recurrent depressive symptoms	89,119	40-80	53.1% female	Individual-level data
PGC MDD 2018[194]	Major depressive disorder	173,005	Adults	Includes males and females	GWAS coefficients
KORA[205]	sgp130 and CRP blood levels	997	32-81	Includes males and females	GWAS coefficients
Framingham[206]	sgp130 blood levels	5,257	Adults	53% female	GWAS coefficients
GTEx[207]	*IL6R* gene expression (blood)	838	21-70	Includes males and females	GWAS coefficients
Westra 2013[209]	*IL6R* gene expression (blood)	5,311	Adults	Includes males and females	GWAS coefficients
CAGE[208]	*IL6R* gene expression (blood)	2,765	Adults	Includes males and females	GWAS coefficients

Table 3.1 shows the samples used in the main analysis and exploratory analyses. All samples were adults and included males and females. With the exception of UK Biobank (for which we had access to individual-level data), all other data sources were GWAS or meta-analysis summary statistics from existing published studies.

Table 3.2: Results from sIL-6R Mendelian Randomization analyses

		UK Biobank Sample			PGC MDD 2018 coefficients		
	Method / SNP selection	Odds ratio (95% CI)	P	# SNPs	Odds ratio (95% CI)	P	# SNPs
van Dongen 2014 coefficients[b]	Ratio of Coefficients (rs12126142)	1.026 (1.009-1.042)	0.002	1	1.014 (1.001-1.027)	0.033	1
	Maximum Likelihood						
	Clumping at r2=0.001	**	**	2	**	**	2
	Clumping at r2=0.01	1.024 (1.008-1.040)	0.004	4	1.015 (1.002-1.027)	0.024	4
	GSMR						
	Clumping at r2=0.05	1.026 (1.010-1.043)	0.001	14	1.015 (1.002-1.028)	0.019	16
	Clumping at r2=0.10	1.026 (1.010-1.042)	0.001	23	1.017 (1.005-1.030)	0.007	23
	Clumping at r2=0.15	1.023 (1.007-1.038)	0.004	26	1.016 (1.004-1.029)	0.009	26
	Clumping at r2=0.20	1.024 (1.008-1.040)	0.002	28	1.016 (1.004-1.028)	0.010	29
	PCA-IVW	1.023 (1.006-1.039)	0.006	491 (4 PCs)	1.016 (1.003-1.029)	0.019	500 (4 PCs)
	PCA-IVW (conditional)*	1.029 (0.994-1.065)	0.107	275 (2 PCs)	1.012 (0.985-1.040)	0.387	280 (2 PCs)
IMPROVE coefficients[a]	Ratio of Coefficients (rs2228145)	1.040 (1.014-1.066)	0.002	1	1.021 (1.002-1.041)	0.032	1
	Maximum Likelihood						
	Clumping at r2=0.001	**	**	2	**	**	2
	Clumping at r2=0.01	1.045 (1.022-1.069)	< 0.001	4	1.023 (1.004-1.041)	0.015	4
	COJO at p=5e-8	1.016 (1.003-1.030)	0.016	7	1.012 (1.001-1.023)	0.026	7
	COJO at p=1e-6	1.018 (1.005-1.032)	0.009	9	1.013 (1.003-1.024)	0.015	9
	COJO at p=0.0001	1.013 (1.002-1.024)	0.017	15	1.009 (1.000-1.017)	0.040	14
	GSMR						
	Clumping at r2=0.05	1.021 (1.004-1.039)	0.016	11	1.008 (0.994-1.022)	0.274	13
	Clumping at r2=0.10	1.023 (1.006-1.040)	0.009	15	1.008 (0.995-1.022)	0.239	17
	Clumping at r2=0.15	1.027 (1.010-1.044)	0.002	22	1.009 (0.996-1.022)	0.197	25
	Clumping at r2=0.20	1.024 (1.008-1.041)	0.003	25	1.012 (0.999-1.025)	0.074	28
	PCA-IVW	1.023 (1.002-1.045)	0.029	519 (7 PCs)	1.021 (1.004-1.038)	0.014	533 (7 PCs)

[a] sIL-6R in units of $1*10^{-8}$ g/mL [b] sIL-6R in units of log pg/mL

* This analysis used coefficients from the van Dongen 2014 paper's Supplementary Table 3, a GWAS of sIL-6R conditional on rs2228145 genotype / ** Not enough SNPs to perform analysis

Table 3.2 shows the results of Mendelian Randomization analyses using two sIL-6R datasets (shown on the left edge) and two outcome datasets (shown across the top). The methods column shows both the analysis method (aligned left) and the SNP selection method (aligned right). For the Maximum Likelihood analysis, LD clumping was performed over a distance of 10,000 kilobases, and for the GSMR analysis clumping was performed using a 1 megabase window. For PCA-IVW analyses, the number appearing in parenthesis after the number of SNPs in the number of principle components (PCs) extracted from the SNP data to explain 99% of the variance in the risk factor.

Table 3.3: Association between classical signaling (using CRP as a proxy) and depression using SNPs known to influence sIL-6R

SNP	Distance (bp) from *IL6R* gene	Relationship to rs2228145		Mendelian Randomization (sIL-6R[a])		Mendelian Randomization (CRP[b], used as a proxy for effect of SNP on classical IL-6 signaling)	
		r^2	|D'|	OR (95% CI)	P	OR (95% CI)	P
rs4129267[a]	In gene	0.996	1.000	1.040 (1.014-1.065)	0.0021	0.761 (0.640-0.905)	0.0021
rs4845623	In gene	0.889	0.977	1.044 (1.016-1.073)	0.0016	0.721 (0.588-0.884)	0.0016
rs4584384	26247	0.351	0.958	1.064 (1.013-1.118)	0.0130	0.443 (0.233-0.842)	0.0130
rs12750774	47027	0.258	0.624	1.045 (1.013-1.079)	0.0058	0.538 (0.346-0.835)	0.0058
rs6691727	27555	0.117	0.935	1.208 (1.031-1.416)	0.0196	0.428 (0.210-0.873)	0.0196
rs9427108	138464	0.066	0.278	1.066 (1.006-1.130)	0.0300	0.674 (0.472-0.963)	0.0300
rs12125166	112679	0.053	0.266	1.075 (1.006-1.150)	0.0329	0.682 (0.480-0.969)	0.0329
rs1395565	206958	0.011	0.234	1.137 (1.040-1.244)	0.0048	0.207 (0.069-0.619)	0.0048
rs10908804	-142336	0.011	0.128	1.082 (1.013-1.155)	0.0183	1.251 (1.039-1.506)	0.0183
rs1194580	-185708	0.000	0.002	1.174 (1.024-1.346)	0.0219	1.336 (1.043-1.711)	0.0219

[a] Using IMPROVE sIL-6R coefficients, units of log pg/mL [b] Using KORA CRP coefficients, units of relative florescence

* rs4129267 was the best-available proxy for rs2228145 when using the KORA CRP coefficient data.

** Negative numbers indicate distance upstream of the gene start, positive numbers indicate distance downstream of the gene end.

Table 3.3 uses SNPs which produced a significant causal effect estimate for the effect of higher sIL-6R on depression when used for single-SNP Mendelian Randomization analysis, and examines whether these SNPs will also produce a significant Mendelian Randomization result for CRP (used as a proxy for the SNP's effect on classical IL-6 signaling). Most of the SNPs analyzed also produced an odds ratio consistent with a protective effect of higher classical IL-6 signaling against depression (or a causal effect of lower classical IL-6 signaling on depression). The two SNPs at the bottom of the Table 3.3 have little to no linkage disequilibrium with rs2228145 and are the only SNPs for which the odds ratios calculated using CRP do not match the effects of rs2228145.

P-values in this table appear similar when rounded to 3 digits because when using the single-SNP Wald ratio with imbalanced sample sizes, the larger sample (UK Biobank n=89,119) will tend to have a stronger effect on the final p-value than the smaller sample (IMPROVE sIL-6R n=3394, KORA CRP n=997).

Table 3.4: Results from PCA-IVW analyses for sIL-6R and depression using SNPs filtered to exclude LD with rs2228145 ($r^2 \leq 0.01$ and $|D'| \leq 0.15$)

	UK Biobank sample			PGC MDD 2018 coefficients		
Exposure sample	Odds ratio (95% CI)	P	# SNPs	Odds ratio (95% CI)	P	# SNPs
van Dongen 2014[a]	0.991 (0.898-1.093)	0.849	13 (2 PCs)	1.109 (1.023-1.202)	0.012	13 (2 PCs)
van Dongen 2014 (conditional)[a]*	0.991 (0.852-1.151)	0.902	72 (2 PCs)	1.157 (1.022-1.311)	0.021	77 (3 PCs)
IMPROVE[b]	1.049 (0.991-1.110)	0.099	89 (4 PCs)	1.046 (0.999-1.095)	0.056	91 (5 PCs)

[a] sIL-6R in units of $1*10^{-8}$ g/mL [b] sIL-6R in units of log pg/mL

* This analysis used coefficients from the van Dongen 2014 paper's Supplementary Table 3, a GWAS conditional on rs2228145 genotype

r^2 refers to squared correlation between each SNP and rs2228145, |D'| refers to Lewontin's D-prime statistic calculated between each SNP and rs2228145

Chapter 4: Examining Competing Explanations for the Depression/Atopy Comorbidity

Introduction

Atopic disorders are a family of conditions characterized by abnormal immune responses to benign stimuli such as pollen. Common atopic disorders include allergic rhinitis, asthma, and eczema, which affect 17-29%, 7-12% and 7-10% of adults, respectively.[142,223–225] A number of cross-sectional and longitudinal studies have reported positive associations between history of atopic disorders and a range of psychiatric disorders.[69,70,226–230 66,231] For example, Chapter 2 of this dissertation found that seasonal allergies are associated with depression, generalized anxiety disorder, panic disorder, and post-traumatic stress disorder. Associations between atopy and psychiatric disorders have been replicated across several continents and ethnicities.[232]

Studies examining shared genetic liability

Heritability studies have suggested an overlap between genetic factors influencing atopy and depression. Twin studies have reported that genetic effects account for 64-77% of the covariance between atopy and depression or internalizing symptoms, with little to no involvement of shared environmental factors.[151,233] Studies using Linkage Disequilibrium (LD) Score Regression to examine genetic correlation using genomic data have also reported significant correlations between asthma and depression,[234,235] anxiety,[235] and neuroticism,[236] and suggestive correlations between allergies and depression,[237] bipolar disorder,[236] and neuroticism.[236] Although heritability and genetic correlation studies can establish that two traits share common genetic risk factors, they cannot characterize the nature of the relationship. Shared genetic risk can result from the effects of genetic variants on a biological pathway common to

both traits, from pleiotropic genes which affect multiple biological pathways, or from genetic influences affecting one trait which in turn affects the other trait (eg. a causal relationship between the traits).[238,239]

Three studies examining the cross-aggregation of atopy and psychopathology with families have reported increased risk of depression, anxiety, or internalizing psychological symptoms in individuals whose first-degree relatives had atopic disorders, regardless of their own atopic status.[152,240,241] The fact that non-atopic relatives of atopic cases are at increased risk for psychiatric disorders is inconsistent with a causal association, since the atopic phenotype cannot have a causal effect on the psychiatric phenotype in non-atopic individuals.[242] However, the low sensitivity of self-reported atopic phenotypes (36%-70% when comparing self-report to clinical testing and diagnosis)[243,244] can impact the validity of this type of study design by leading to misclassification of relatives with atopy as non-atopic.

Potential causal relationships

Another important hypothesis to examine is the potential for a causal effect of atopic disorders on depression. Plausible mechanisms through which atopic disorders may contribute to depressive symptoms include increases in inflammatory signaling,[69,245,246] disturbances in sleep,[247] and discomfort and stress caused by the atopic symptoms.[71] Additional findings suggestive of a causal effect include seasonal variations in depressive symptoms among individuals with pollen allergies[19,246] and a lower prevalence of depression in individuals whose allergies were treated with desensitization therapies.[248]

An influence of depression on atopic symptoms may also be possible due to the effect of stress on the immune system.[249] Another recent Mendelian Randomization study has reported an apparent causal effect of depression on asthma,[198] however that study may have experienced bias due to the use of overlapping samples.[250] A crossover study comparing allergen skin test responses under different conditions found that on days during which participants were assigned to complete a stressful task, they experienced larger skin reactions and reacted to a greater number of allergens than on control days.[73,74] One randomized clinical trial has also reported improvements in allergic symptoms among individuals being treated with antidepressants.[251] Although in some ways such a relationship might be regarded as a causal effect of stress or

depression on atopy, depression could also be conceptualized as an influence which lowers the threshold for genetic liability required to produce an atopic response, while not necessarily having a direct causal effect on atopy.[252]

Other possible explanations

A final explanation suggested in existing literature is that the association between atopy and psychiatric disorders is a spurious association resulting from somatization (the perception of psychological distress as physical symptoms) or from differences in atopic disorder self-diagnosis and self-reporting by individuals with psychiatric disorders. This hypothesis is supported by a study which found that anxiety disorders were associated with self-reported allergies but not objectively-verified allergies,[75] and another study reporting that adjustment for somatization significantly attenuated the relationship between depression/anxiety and eczema.[76,253] This hypothesis may also provide an alternative explanation for the improvement in allergic symptoms observed among some individuals treated with antidepressants.[251] Evidence opposing this hypothesis includes replication of the association between depression and allergies in studies where allergic status was verified using skin-prick tests or blood tests,[254,255] and validation studies reporting relatively high specificity for questions asking about "doctor-diagnosed" asthma, allergic rhinitis, and eczema (99%, 93%, and 95% respectively).[243,244]

Goal of the present study

In sum, there is a large body of evidence documenting the co-occurrence of depression with a range of atopic disorders, but the reasons for this relationship are not yet established. The purpose of this study is to examine various competing explanatory models for the comorbidity between depression and three common atopic disorders (i.e., allergic rhinitis, eczema, and asthma) using a variety of approaches and methods. First, LD Score Regression[238] will be used to examine genetic correlation and co-heritability. Next, Mendelian Randomization[100] will be used to examine the potential causal effect of atopy on depression. Finally, comparison of atopy polygenic risk scores will be used to examine the possibility of a spurious relationship, or of lowering of the liability threshold for atopic responses among individuals with depression. These

analyses will be conducted using data from the UK Biobank, a large genetic cohort that has detailed data on both mental health and atopic phenotypes, supplemented with genetic association summary statistics from previously published sources.

Methods

Data sources and phenotypes

UK Biobank data and phenotypes

The primary data source for this study is the UK Biobank, a large cohort of more than 500,000 adults in the United Kingdom.[256] Participants in the UK Biobank were genotyped using Affymetrix genotyping arrays, with additional genetic variants imputed using data from the Haplotype Reference Consortium, the UK10K panel, and the 1000 genomes phase 3 data.[222,257,258,258] Details of the overall study design of UK Biobank are described in existing publications.[259] Further details regarding eligibility criteria, phenotyping, and GWAS procedures used with the UK Biobank data in the present study are included in the supplemental note. Details of all samples and data sources used in this study are shown in Table 4.1.

We defined the phenotype "Recurrent Depressive Symptoms" as a lifetime history of experiencing a cardinal symptom of depression (low mood or anhedonia)[2] on at least two occasions. We classified participants as controls if they did not report any periods during which they experienced cardinal symptoms of depression. Because questions about lifetime history of depression were only included during the final two years of the UK Biobank intake period,[260] we included data from two other UK Biobank questionnaires to produce a larger sample size.

We defined the atopic disorder phenotypes based on an intake interview question that asked participants "Has a doctor ever told you that you have had any of the following conditions?" and included "Asthma" and "Hay fever, allergic rhinitis, or eczema" as possible responses. From this self-report data we defined the phenotypes "Asthma" and "Allergies/Eczema". In analyses where methods required non-overlapping samples (e.g. Mendelian Randomization and Polygenic Risk Score comparison), we split the UK Biobank into a *Complete Depression Data* sample (n=127,271) consisting of individuals who had completed

one or more of the depression questionnaires, and an *Atopic Phenotypes Only* sample (n=163,645) consisting of individuals who had insufficient data for evaluating lifetime history of depression (and thus their lifetime depression phenotype was unknown). This approach allowed us to retain the largest number of observations across the non-overlapping samples. Separate GWAS were conducted for each UK Biobank phenotype/sample combination used throughout the analyses, eg. GWAS for the Allergies/Eczema phenotype were conducted using the full UK Biobank sample (for LD Score Regression), the *Atopic Phenotypes Only* sample, and additional sub-divided samples required for the PRS analysis.

Summary statistics from published GWAS meta-analyses

In addition to the data and phenotypes described above, we also used three sets of summary statistics from published GWAS meta-analyses. We used coefficients from Waage et al 2018[79] for the phenotype "Allergic Sensitization", which refers to an allergy phenotype defined by a positive skin prick test or blood test result for one or more common allergens. Although allergic sensitization is an objectively-measured atopic phenotype, it is important to note that it does not directly correspond to other atopic phenotypes, as some allergically-sensitized individuals experience no symptoms, while some symptomatic individuals may test negative if the allergens they are sensitive to are not included in the test.[261] We used GWAS coefficients from the Paternoster et al 2015 GWAS of atopic dermatitis,[262] to examine the phenotype of "Atopic Dermatitis," which is a common cause of eczema. Finally, we used GWAS coefficients from the Psychiatric Genomics Consortium 2018 meta-analysis of Major Depressive Disorder (PGC MDD 2018)[194] to examine the phenotype "Major Depressive Disorder" defined as depressive symptoms that met a set of clinical diagnostic criteria such as the DSM-V,[2] the ICD-10,[263] or earlier versions of either definition.

Analyses

Multiple analyses were used to examine the different potential explanations for the depression/atopy comorbidity. A graphical overview including information about each hypothesis and the methods used to examine it is shown in Figure 4.1. Further details regarding hypothesis and methods are available in Supplemental Table D.7.

(1) Analysis of Genetic Correlation and Co-Heritability of Atopic and Depression phenotypes

The goal of this analysis was to assess the similarity in the genetic factors contributing to atopy and to depression analyzed by the cross-trait genetic correlation (e.g., recurrent depressive symptoms and asthma) using LD Score Regression. Coefficients from GWAS results were harmonized to use the same effect alleles and prepared for analysis using scripts provided by the LD Score Regression authors.[264] Coefficients coming from meta-analyses were additionally filtered for SNP-specific sample size and between-cohort heterogeneity, and described in Table D.1. Coefficients were then analyzed using LD Score Regression with the 1000 Genomes phase 3 European sample[257] used as a reference sample for linkage disequilibrium (LD). We examined two related but distinct measures of the shared genetic influences on atopy and depression: genetic correlation and co-heritability.

Genetic correlation is defined as the genetic covariance between two traits (σ_{g_x,g_y}) divided by the square root of the product of their genetic variances, as shown in Equation 4.1.[265] Because both the numerator and denominator relate to genetic variance, genetic correlation provides a measure of how genetic factors influencing each trait are related to each other. This allows genetic correlation to be high even when the correlated genetic influences make only a small contribution to the co-occurrence of the two traits. Co-heritability is defined as the genetic covariance between two traits divided by the phenotypic covariance between two traits (σ_{p_x,p_y}), as shown in Equation 4.2.[266] Co-heritability provides a measure of the extent to which the co-occurrence of two traits can be attributed to genetic factors, and is influenced by the overall heritability of each trait. For co-heritability, we used the estimated population prevalences in Table D.1 to convert from the observed scale (which is dependent on case/control proportions in the sample) to the liability scale (which is adjusted to reflect disease prevalence in the general population).[95] Because each pairing of phenotypes was treated as a separate test in this analysis (e.g., correlations between the two depression phenotypes or between different atopic phenotypes were also considered as outcomes of interest), we calculated a Bonferroni-corrected p-value threshold of 0.003 (0.05 / 15 phenotype pairs).

Equation 4.1: Genetic correlation

$$r_g = \frac{\sigma_{g_x,g_y}}{\sqrt{\sigma^2_{g_x}\sigma^2_{g_y}}}$$

Equation 4.2: Co-heritability

$$h_{x,y} = \frac{\sigma_{g_x,g_y}}{\sigma_{p_x,p_y}}$$

LD Score Regression works by regressing the χ^2 value for each SNP (χ^2_j) on an LD score for each SNP (ℓ_j), which is a measure of the genetic variation tagged by each SNP (further details are given in Bulik-Sullivan 2015).[264] Under polygenic inheritance, SNPs tagging larger numbers of other SNPs are more likely to be in LD with a causal variant and thus have a higher χ^2 value.[264] Inflation of χ^2 values due to LD with causal SNPs will be captured in the LD Score Regression slope, while inflation from other sources (such as population genetic stratification) will be uncorrelated with LD and will be captured by the intercept.[264] In cross-trait LD Score Regression, χ^2 values are replaced with products of SNP z-scores for each trait (i.e., $z_{x_j}z_{y_j}$ for the z-scores of SNP j with traits x and y). Because χ^2 values are the squares of z-scores, the resulting quantity behaves similarly to a χ^2 value, but is negative for SNPs having associations of opposite signs with the two traits. The formula for cross-trait LD Score Regression is given in Equation 4.3, where N_x and N_y are sample sizes for traits x and y, M is the number of SNPs, N_s is the number of overlapping individuals between the two samples, and ρ is the correlation between phenotypes in the overlapping individuals.[238] The estimated slope is then solved for an estimate of genetic covariance ($\hat{\sigma}_{g_x,g_y}$), which can then be used to estimate genetic correlation and co-heritability.

Equation 4.3: Cross-trait LD Score Regression

$$E\left[z_{x_j}z_{y_j}\middle|\ell_j\right] = \frac{\sigma_{g_x,g_y}\sqrt{N_xN_y}}{M}\ell_j + \frac{\varrho N_s}{\sqrt{N_xN_y}}$$

The high levels of LD in the Major Histocompatibility Complex (MHC, a dense region of immune-related genes located on chromosome 6)[267] have the potential to result in high-leverage outliers that bias LD Score Regression results.[238,268] As a result, this region is often excluded during LD Score Regression analysis. However both atopic[269] and depressive[194] phenotypes have known genetic associations in this region, and thus excluding the MHC could potentially lead to under-estimation of genetic correlation between traits. To address this issue, we conducted an

additional analysis to assess how inclusion of the MHC region impacted genetic correlation and co-heritability estimates.

(2) Mendelian Randomization to Assess the Causal Impact of Atopy on Depression

We used two-sample Mendelian Randomization to assess the possibility of a causal effect of each atopic phenotype (allergies/eczema, asthma, allergic sensitization, atopic dermatitis) on the two depression phenotypes (Recurrent Depressive Symptoms in the UK Biobank and Major Depressive Disorder from PGC MDD 2018).

Overlap between the exposure sample and the outcome sample can create bias in two-sample Mendelian Randomization studies.[250] To avoid this bias, we used the *Atopic Phenotypes Only* sample for each UK Biobank atopic phenotype (which excludes all individuals with data on lifetime depression history) when calculating GWAS coefficients for use in Mendelian Randomization. We also excluded individuals born in March of 1958 from the UK Biobank sample to prevent overlap with the British 1958 birth cohort, a sample included in both Paternoster 2015 and Waage 2018. Further details regarding prevention of overlap are available in the Supplementary Note for this chapter.

We used Plink 1.90[270] to clump significant ($p < 5x10^{-8}$) SNPs from the atopic disorder coefficients (r^2=0.001, distance=10,000 kb), resulting in a set of independent genetic variants for each atopic exposure. We used these SNPs to perform Mendelian Randomization using the two sample maximum likelihood method, in which information from multiple independent SNPs is combined to produce a causal effect estimate. We conducted this analysis using R 3.6.0 and TwoSampleMR 0.5.4.[271] We also repeated each analysis using Generalized Summary-based Mendelian Randomization (GSMR),[198] a method capable of accounting for partial LD to include a greater number of SNPs in the analysis. As a result, the GSMR approach typically has greater statistical power than methods based on independent SNPs.[198]

To account for multiple comparisons, we used a Bonferroni-corrected p-value threshold of 0.0125 to account for the four atopic exposures examined. Additional tests intended to examine consistency across Mendelian Randomization methods or replication across different datasets were not included in the Bonferroni calculation.

Finally, while of scientific interest, we did not attempt to use Mendelian Randomization to assess the causal effect of the depressive phenotypes on atopic disorder phenotypes. We feel this analysis is not appropriate at this time because the biological relationships between genetic variants and depression symptoms are complex and likely to involve mechanisms that also impact other traits. Depression has been described as a "watershed" phenotype in which a genetic variant with a particular "upstream" effect on brain biology may have dozens of "downstream" effects that in turn contribute to depression and numerous other phenotypes.[272] This makes it difficult to confirm whether depression-associated variants meet the Mendelian Randomization requirement that "the effect of the genetic instrument on the outcome must be mediated exclusively by the exposure in question."[273] Without this assumption being met, it would not be appropriate to interpret the results of a Mendelian Randomization analysis as indicative of a causal effect.

(3) Polygenic Risk Score Analysis to Explore Other Explanatory Models of the Relationship between Atopy and Depression

The objective of the polygenic risk score (PRS) analysis was to assess whether genetic liability for atopy, indicated by PRSs for atopic disorders, differs between atopy cases with and without Recurrent Depressive Symptoms. While this analysis is exploratory, the results are useful for informing future research. For example, if the genetic liability for atopy is lower for atopic cases who also have Recurrent Depressive Symptoms, this is consistent with the notion that people with depressive symptomatology are more likely to self-report or seek care for atopic symptoms (i.e., differential self-reporting or somatization). Alternatively, it is also consistent with the notion that having a history of depression reduces the threshold at which genetic liability towards atopy is manifested phenotypically.

PRS for atopy were calculated using PRSice-2, which uses two data sources for score calculation.[274] The first sample, called the *base sample*, is used for estimation of coefficients for associations between each SNP and the outcome of interest. Base sample coefficients are provided as GWAS summary statistics. The second sample, called the *target sample*, is used to identify the optimal p-value cutoff for SNP inclusion in the score to maximize the percent of the variance in the trait that can be explained by the PRS. To avoid inclusion of multiple SNPs in LD

with the same causal variant, PRSice-2 clumps SNPs for LD (r^2=0.10, distance=250kb) prior to evaluating score performance in the target sample. Once developed, the resulting score can then be applied to one or more *analytic samples* which do not contain individuals from the base or target samples used to create the score.

The *Allergic Sensitization* score used base sample data from Waage 2018, a GWAS for allergic sensitization, and used the *Atopic Phenotypes Only* UK Biobank Allergies/Eczema sample as the target sample. We used an objectively-measured atopic phenotype (i.e., allergic sensitization measured by blood test or skin prick test) as the base sample for this PRS to reduce the influence of any biases affecting self-reported atopic phenotypes. The use of a self-reported phenotype for the target sample is unlikely to bias the PRS, because this sample is used only to select a p-value threshold for SNP inclusion.[274]

Because the strength of a PRS is strongly influenced by the size of the base sample,[275] we also developed two additional PRSs using UK Biobank (UKB) data for the Allergies/Eczema phenotype. For the *UKB large* PRS, we used participants from the *Atopic Phenotypes Only* Allergy/Eczema sample (eg. individuals who lacked data necessary for phenotyping of lifetime history of Recurrent Depressive Symptoms). For the *UKB strict* PRS, we further restricted the sample to individuals who had reported no depressive symptoms in the two weeks preceding the UK Biobank intake interview. For both PRSs, we randomly selected 8000 eligible participants (4000 Allergy/Eczema cases and 4000 controls) to exclude from the base sample for use as a target sample. After creation, we confirmed that each PRS was normally distributed, and scaled each PRS to have a mean of 0 and a standard deviation of 1 to improve comparability across analyses using different scores.

After creating the PRSs, we applied them to individuals in the analytic sample (phenotyped cases and controls for the Recurrent Depressive Symptoms phenotype). We confirmed the ability of the PRS to predict the Allergies/Eczema phenotype in the UK Biobank depression sample, then compared the PRS values for individuals with self-reported Allergies/Eczema to assess whether the scores differed between Allergy/Eczema cases with and without depression. To account for multiple comparisons, we calculated a lenient Bonferroni-corrected p-value threshold of 0.017 (0.05 / 3 scores) and a strict threshold of 0.006 (0.05 / 9 comparisons between mean scores).

Results

The genetic correlations between phenotypes are shown in Table 4.2. The estimated co-heritabilities are shown in Table D.2. As expected, there were high levels of genetic correlation between pairs of atopic phenotypes (e.g., r_g=0.686, p=6.4e-133 for Allergies/Eczema and Asthma), and between the two depression phenotypes (Recurrent Depressive Symptoms and Major Depressive Disorder, r_g=0.856, p=1.3e-127) attesting to the comparability of different phenotype definitions in the different samples.

Analysis of Genetic Correlation and Co-Heritability

Both depression phenotypes had significant genetic correlations with Asthma (Recurrent Depressive Symptoms: r_g=0.171, p=1.0e-06, Major Depressive Disorder: r_g=0.236, p=2.0e-12). Only the recurrent depressive symptoms phenotype had a significant genetic correlation with the allergies/eczema phenotype (r_g=0.178, p=3.9e-08), and neither depression phenotype had a significant genetic correlation with Allergic Sensitization or Atopic Dermatitis. Co-heritability estimates, shown in Table D.2, ranged from 25.4% (SE 4.6% for Recurrent Depressive Symptoms and Allergic Sensitization) to 10.3% (SE 0.6%, for Recurrent Depressive Symptoms and Major Depressive Disorder). These co-heritability estimates can be interpreted as the percent of the phenotypic covariance explained by genetic covariance, eg. 25.4% of the covariance between Recurrent Depressive Symptoms and Major Depressive Disorder can be explained by shared genetic influences. When we repeated this analysis including the MHC (Tables D.3 and D.4), estimates for r_g became slightly smaller, but the statistical significance of the associations between depression and atopy did not substantively change. Across all analyses, previously-significant results remained significant after accounting for multiple comparisons, even at the Bonferroni-corrected p-value threshold of 0.003.

Mendelian Randomization to Assess the Causal Impact of Atopy on Depression

The results of the Mendelian Randomization analyses are shown in Table 4.3. Across all atopy exposures and both depression outcomes the odds ratio effect estimates were near 1.0, and

there were no statistically significant associations after adjustment for multiple comparisons. There was one nominally-significant association (between Atopic Dermatitis and Major Depressive Disorder, Odds ratio: 1.034, 95% CI: 1.000-1.069, p=0.047) identified using GSMR; however, this association did not replicate using the other depression phenotype, nor did it remain consistent when using a different Mendelian Randomization method. There was significant heterogeneity among the instrumental SNPs detected in some analyses, which could reduce the power to detect significant results, and in some cases could indicate violations of Mendelian Randomization assumptions. However, upon inspection of SNP forest plots (Figures D.1 and D.4) there were no extreme outliers. Finally, the findings in Table 4.3 were substantively unchanged after exclusion of individual SNPs with the largest differences from the mean estimate, as shown in Supplemental Figures D.2 and D.5.

Polygenic Risk Score Analysis

Details regarding the performance of each PRS are shown in Table D.6. All three scores had highly significant associations with the Allergies/Eczema phenotype in both the target and analytic samples, although the percent of variance in the trait explained by each score was relatively low (R^2: 0.005 for the *Allergic Sensitization* score, 0.015 for the *UKB strict* score, and 0.018 for the *UKB large* score). The *UKB large* score had a weak but significant association with depression in the analytic sample (OR: 1.01, 95% CI: 1.00 - 1.03, P: 0.0257), which may reflect the higher proportion of Allergy/Eczema cases among individuals with Recurrent Depressive Symptoms (28.3% in depression cases and 21.3% in controls).

Results for the comparison of the PRS by depression and atopy status are shown in Table 4.4. For the *Allergic Sensitization* score, the mean scaled score was slightly lower among Allergy/Eczema cases with a history of Recurrent Depressive Symptoms than among those with no depression history (0.099 vs. 0.123, p=0.0407). The differences by depression status are relatively small compared to the differences by Allergy/Eczema status, as shown in Figure 4.2. For the *UKB large* score, Recurrent Depressive Symptoms cases had a significantly higher mean scaled score than controls (0.011 vs. -0.002, p=0.0257), but this pattern reversed when mean scores for Recurrent Depressive Symptoms cases and controls were compared only among individuals who reported Allergies/Eczema (cases: 0.182, controls: 0.202, p=0.0886). However,

none of these differences remained statistically significant after adjustment for multiple comparisons even at the more lenient Bonferroni-corrected p-value threshold of 0.017.

Discussion

Our findings indicate significant shared heritability and genetic correlation between depression and some atopic phenotypes, but do not support a causal effect of atopy on depression. Among individuals with Allergies/Eczema, genetic liability for atopic traits as indicated by various PRSs, tended to be slightly lower among those who also had a history of depression, but these differences were small and did not persist after accounting for multiple comparisons.

Genetic Correlation of Atopy and Depression

The results showing significant genetic correlation between the depression phenotypes and asthma are consistent with existing results from LD Score Regression[234,235] and twin studies.[151] The significant genetic correlation between Recurrent Depressive Symptoms and Allergies/Eczema in the UK Biobank sample is also consistent with previous literature;[151,237] however, this correlation did not replicate when using the PGC MDD 2018 depression phenotype, nor when comparing the UK Biobank sample to the Waage 2018 Allergic Sensitization phenotype. Nevertheless, a genetic correlation between depression and allergic phenotypes remains highly plausible in light of the known genetic correlation between depression and asthma and the high genetic correlations between the different atopic phenotypes themselves.[237]

It is important to note that all non-UK Biobank coefficients used in this analysis came from meta-analyses, which tend to produce lower estimates in LD Score Regression due to higher heterogeneity in meta-analysis samples.[276] Additionally, the Waage 2018 meta-analysis applied genomic control correction, which can bias LD Score Regression results by suppressing test statistic inflation related to linkage disequilibrium.[268] However, we still found high levels of genetic correlation between phenotypes within the same category (e.g., between our UK Biobank Recurrent Depressive Symptoms phenotype and the PGC MDD 2018 phenotype), indicating

these phenotypes and samples had substantial “signal.” Further replication using additional datasets will help clarify and quantify the genetic coheritability between depression and atopy phenotypes.

Mendelian Randomization of Atopy on Depression

Although the results of the Mendelian Randomization analysis do not support a causal relationship between atopy and depression, it is important to note that depression is a highly heterogeneous disorder, and thus it remains possible that atopy may play a contributing role in at least some cases of depression. Symptoms of atopic disorders such as asthma attacks or large rashes can cause anxiety, embarrassment, and distress in individuals who experience them, and this impairment in quality of life has the potential to contribute to depression in at least some individuals.[277,278] Regardless of whether the relationship between atopy and depression is causal, it is important for clinicians to recognize that individuals with atopy are at increased risk for depression, and may benefit from more frequent screenings and discussions regarding mental health concerns.

Polygenetic Risk Score Analysis

While the PRS analysis was exploratory in intent, and the differences detected did not remain significant after adjustment for multiple comparisons, these findings still identified interesting avenues for future research. While only nominally statistically significant, this analysis found that among individuals with self-reported atopy, those with depression tend to have lower atopy genetic liability. If replicated by future work, there are several potential explanations for this finding. For example, this finding may reflect a higher proportion of "false positive" self-report atopy among individuals with depression, with individuals reporting atopic symptoms potentially due to somatization or to differences in self-diagnosis. However, even if such misclassification were to occur, it is unlikely to be the sole explanation for the association between depression and atopy, as this association has been reported even in studies which used objective measures of atopy such as allergen skin-prick testing.[254,255]

The difference in PRS could also suggest that the presence of depression (or of a common risk factor associated with depression, such as stress) may result in increased susceptibility to atopic responses that could allow this trait to manifest even in individuals with lower genetic liability. This explanation is consistent with existing studies that have reported exacerbated atopic responses and responses to a greater number of antigens in individuals who have completed a stressful task.[73,74] Future studies should build on these findings to clarify the relative importance of these completing explanations.

Strengths and limitations

Findings should be interpreted in light of study limitations and strengths. One important limitation is the reliance on self-reported data for most atopic phenotypes, which is known to have fairly poor sensitivity,[243,244] resulting in likely misclassification of many atopy cases as controls. Misclassification of cases as controls can increase the similarity of the control group to the case group, potentially reducing statistical power to detect differences between the groups. It should also be noted that the PRS analysis produced only a modestly significant result that was not significant after adjustment for multiple comparisons, despite using a large sample. Additionally, as shown in Figure 4.2, the difference detected between Allergy/Eczema cases with and without depression is extremely small when compared to the difference between Allergy/Eczema cases and controls. "Over-powered" studies with large samples have the potential to detect statistically significant differences that are too small to have clinical or scientific relevance,[279] so while these findings may suggest interesting avenues for future research, their usefulness on their own is limited.

This study also has several strengths, including the use of large, population-based samples, a range of phenotypes, and checks for consistency across methods and replication across samples and datasets to confirm the robustness of the results. Another strength of this study is the inclusion of the objectively-measured atopic phenotype Allergic Sensitization for comparison to self-reported atopic phenotypes in most analyses. Finally, this study employed multiple genetically-based methods, including Mendelian Randomization, LD Score Regression and PRS, to explore the relationship between depression and atopy in a more rigorous manner than could be obtained by using any one of these methods alone.

Conclusions

The relationships between depression and atopy are complex and multi-faceted. The results of this study support an explanation of shared genetic liability between depression and some atopic disorders, while neither strongly supporting nor conclusively eliminating other explanations which may also apply to this relationship. Taken together, the findings from this chapter provide a more complete understanding of the salience of various explanatory models of the relationships between depression and atopy.

Tables and Figures

Table 4.1: Characteristics of samples

Phenotype	Source	N	Age range	% Female	Described Ancestry	Notes
Recurrent Depressive Symptoms	UK Biobank	127,271	39-80	52.2%	100% white British	*Complete depression data sample:* All eligible individuals with data necessary for phenotyping of lifetime history of recurrent depressive symptoms
Major Depressive Disorder	PGC MDD 2018 Wray 2018[194]	173,005	Adults	Includes both males and females	100% European ancestry	GWAS summary statistics, selected for use as a second depression sample to confirm results replicate across samples
Allergies/eczema	UK Biobank	331,664[a]	39-73	53.8%	100% white British	*Full sample*: All eligible UK Biobank participants *Atopic phenotypes only sample*: Excludes individuals eligible for the "complete depression data" sample to prevent sample overlap.
Asthma	UK Biobank	331,664[a]	39-73	53.7%	100% white British	*Full sample*: All eligible UK Biobank participants *Atopic phenotypes only sample*: Excludes individuals eligible for the "complete depression data" sample to prevent sample overlap.
Allergic sensitization	EAGLE Waage 2018[79]	23,408	Both adults and children	Includes both males and females	Predominantly Caucasian	GWAS summary statistics, selected due to use on an objectively-measured atopic phenotype
Atopic dermatitis	EAGLE Paternoster 2015[262]	173,166	Both adults and children	Includes both males and females	100% European ancestry	GWAS summary statistics, selected to examine eczema or atopic dermatitis on its own, rather than as part of the UK Biobank "allergies/eczema" phenotype

[a] N=163,645 when this phenotype is used with the *Atopic Phenotypes Only* sample.
PGC: Psychiatric Genomics Consortium. EAGLE: EArly Genetics and Lifecourse Epidemiology consortium.

Table 4.1 shows the samples used in this chapter. All samples were adults and included males and females. With the exception of UK Biobank (for which we had access to individual-level data), all other data sources were GWAS or meta-analysis summary statistics from existing published studies.

Table 4.2: Genetic correlations between phenotypes

	Recurrent Depressive symptoms	**Allergies/eczema**	**Asthma**	**Major Depressive Disorder**	**Allergic sensitization**	**Atopic dermatitis**
Data source	UK Biobank (complete depression data sample)	UK Biobank (full sample)	UK Biobank (full sample)	PGC	EAGLE	EAGLE
Recurrent Depressive symptoms	--	0.178 (p=3.9e-08)	0.171 (p=1.0e-06)	0.856 (p=1.3e-127)	-0.107 (p=0.112)	-0.050 (p=0.579)
Allergies/eczema	0.178 (p=3.9e-08)	--	0.686 (p=6.4e-133)	0.009 (p=0.791)	0.765 (p=3.4e-36)	0.517 (p=1.4e-13)
Asthma	0.171 (p=1.0e-06)	0.686 (p=6.4e-133)	--	0.236 (p=2.0e-12)	0.572 (p=7.0e-18)	0.399 (p=2.7e-06)
Major Depressive Disorder (PGC)	0.856 (p=1.3e-127)	0.009 (p=0.791)	0.236 (p=2.0e-12)	--	-0.025 (p=0.672)	0.071 (p=0.362)
Allergic sensitization (EAGLE)	-0.107 (p=0.112)	0.765 (p=3.4e-36)	0.572 (p=7.0e-18)	-0.025 (p=0.672)	--	0.436 (p=0.002)
Atopic dermatitis (EAGLE)	-0.050 (p=0.579)	0.517 (p=1.4e-13)	0.399 (p=2.7e-06)	0.071 (p=0.362)	0.436 (p=0.002)	--

Table 4.2 shows results for genetic correlations between depressive and atopic phenotypes, with the Major Histocompatibility Complex excluded. Co-heritability estimates are available in Table D.2, and genetic correlations and co-heritabilities calculated with inclusion of the Major Histocompatibility Complex are provided in Tables D.3 and D.4.

Table 4.3: Mendelian Randomization of the effect of atopic disorders on depression

		Depression phenotypes (outcomes)					
		Depressive Symptoms			Major Depressive Disorder (PGC)		
		Odds ratio (95% CI)	P	# SNPs	Odds ratio (95% CI)	P	# SNPs
Atopic phenotypes (exposures)	Allergies/eczema						
	Maximum Likelihood	0.992 (0.951-1.035)	0.708	35	0.982 (0.940-1.025)	0.401	33
	GSMR	0.992 (0.956-1.029)	0.677	98	0.998 (0.962-1.036)	0.923	82
	Asthma						
	Maximum Likelihood	0.992 (0.957-1.029)	0.679	30[a]	0.980 (0.946-1.015)	0.252	29
	GSMR	0.986 (0.956-1.017)	0.378	84[b]	0.993 (0.963-1.024)	0.643	74[b]
	Allergic sensitization (EAGLE)						
	Maximum Likelihood	1.006 (0.967-1.045)	0.778	10	1.024 (0.985-1.063)	0.233	10
	GSMR	1.005 (0.970-1.041)	0.803	13	1.024 (0.989-1.060)	0.177	15
	Atopic dermatitis (EAGLE)						
	Maximum Likelihood	0.993 (0.951-1.037)	0.075	11[a]	1.030 (0.991-1.072)	0.136	12[a]
	GSMR	0.987 (0.954-1.021)	0.438	23	1.034 (1.000-1.069)	0.047	23

[a] Significant Cochran's Q test for heterogeneity, see notes under Figures D.2 and D.5.
[b] Potentially-pleiotropic SNPs were detected and excluded during HEIDI outlier test, see notes under Figures D.3 and D.6.

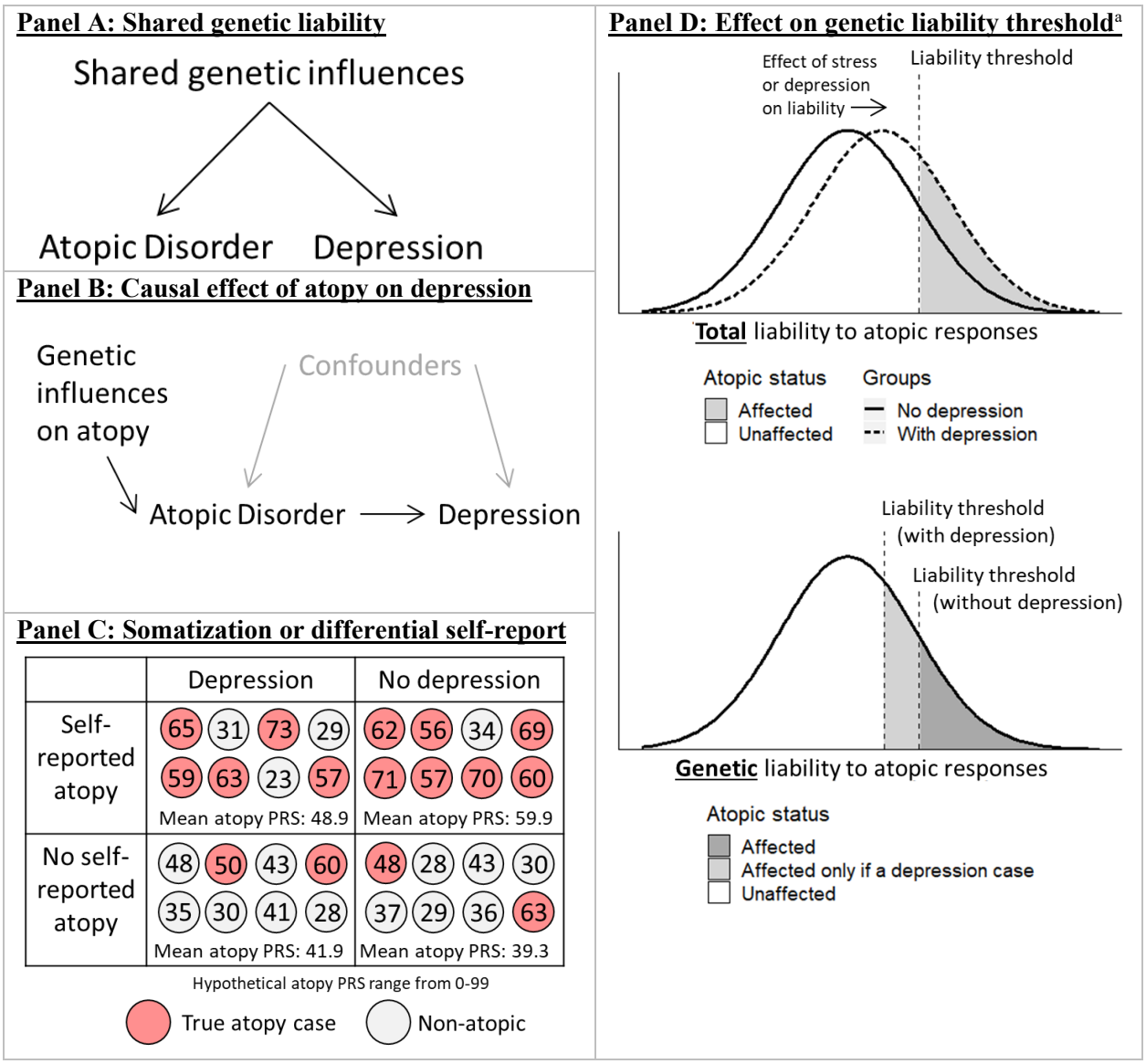
Table 4.3 shows the results of Mendelian Randomizations for the effect of atopic disorders on depression. No results remained significant after adjustment for multiple comparisons.

Table 4.4: Comparisons of atopy polygenic risk scores between Allergy/Eczema cases with and without Recurrent Depressive Symptoms

Score	Scaled polygenic risk score (mean, SD)				Depression cases vs. depression controls		
	Allergy/eczema cases		Allergy/eczema controls		T-test p-values		
	With depression	Without depression	With depression	Without depression	All	Individuals with allergies/eczema	Individuals without allergies/eczema
UKB large	0.182 (0.971)	0.202 (0.991)	-0.056 (0.982)	-0.057 (0.984)	0.0249	0.0886	0.9205
UKB strict	0.113 (0.994)	0.120 (0.995)	-0.046 (0.992)	-0.044 (0.996)	0.2062	0.5025	0.7066
Allergic sensitization	0.099 (0.998)	0.123 (1.016)	-0.037 (0.995)	-0.034 (0.995)	0.7256	0.0407	0.6667

Table 4.4 shows comparisons of mean atopy polygenic risk scores by atopy and depression status. Although there is a tendency for self-reported allergy/eczema cases with depression to have lower mean polygenic risk scores than self-reported allergy/eczema cases without depression, this difference is not statistically significant after adjustment for multiple comparisons.

Figure 4.1: Graphical overview of hypothesis examined in each analysis



[a] Figured inspired by Figure 1 from Werling and Geschwind (2013)[280]

Figure 4.1 illustrates the differing hypotheses for the frequent co-occurrence of depression and atopy. ***Panel A*** illustrates shared genetic liability, in which the same genetic influences contribute to the development of both traits. This hypothesis is examined using LD Score Regression. ***Panel B*** illustrates a causal effect of atopy on depression. This hypothesis is examined using Mendelian Randomization, in which genetic variants uncorrelated with confounders of the depression/atopy relationship are used as instrumental variables for the atopic disorder. ***Panel C*** illustrates a scenario in which either somatization or differential self-reporting of atopic status leads to the group of depression cases with self-reported atopy having a lower proportion of true atopy cases. This hypothesis is examined using polygenic risk score comparison, because a higher proportion of atopy non-cases in this group might be expected to result in a lower group mean polygenic risk score for atopy. ***Panel D*** illustrates a scenario in which depression increases liability to atopic responses (possibly by way of effects on immune regulation), allowing individuals with lower genetic liability to atopy to develop atopic phenotypes. This hypothesis is also examined using polygenic risk score comparison, because it might also be expected to result in a lower mean atopy polygenic risk score among atopic individuals with depression than among atopic individuals without depression.

Figure 4.2: Comparison of Allergic Sensitization PRS among individuals with and without Recurrent Depressive Symptoms

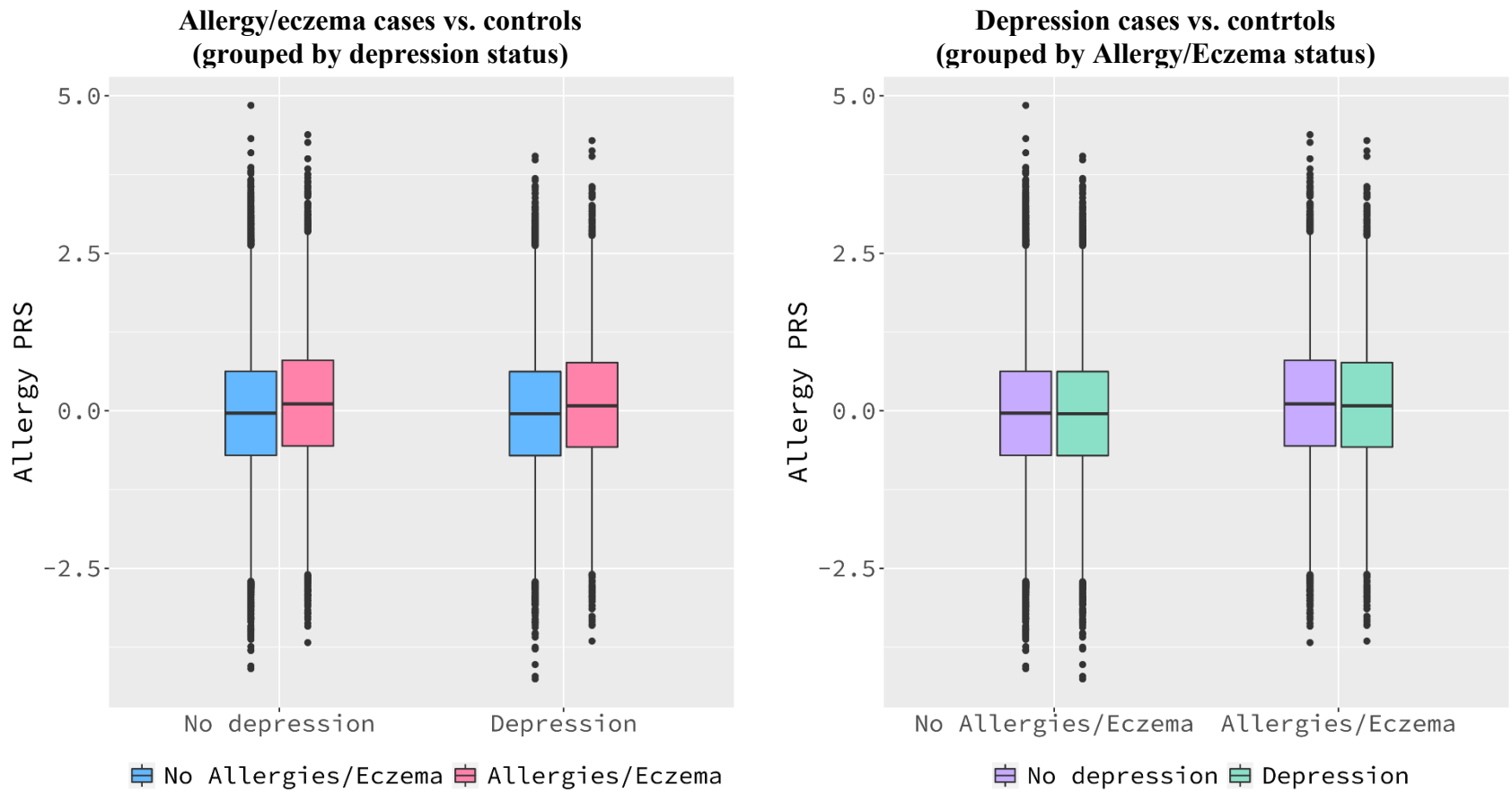


Figure 4.2 shows the distribution of scaled allergic sensitization polygenic risk scores by depression and Allergies/Eczema status. Although the analysis detected a statistically significant difference between Allergies/Eczema cases with vs. without depression (the two pink boxes in the left panel, or the purple/green boxes furthest to the right in the right panel), this difference is not large enough to be easily visible. Additional figures for the other polygenic risk scores are shown in Figure D.7.

Chapter 5: Public Health Impact and Future Directions

Understanding the Impact of Depression: Brain and Body, Individuals and Populations

Depression is a serious mental disorder with far-reaching effects on the lives of affected individuals, their families, and society. The most direct impact of depression is the symptoms it causes the affected individual, such as low mood, fatigue, loss of interest or pleasure in life activities, and sleep disturbances.[2] Although this mental suffering can be difficult to observe or quantify, it can have a profound impact on the individual's life, and can cause substantial impairment and disability.[281] When depression impacts an individual's ability to function in the roles that are expected of them, this can also impact others around them. Children of parents with depression are at increased risk for mental disorders and poor school performance.[282,283] Depression can lead to more frequent absences from work, and to impaired performance or "presenteeism" in the workplace.[284] Individuals with depression are also more likely to experience job loss, and less likely to find employment.[285,286] Individuals with depression are also at increased risk for substance abuse and addiction.[287] Finally, depression is a known risk factor for suicidal ideation and suicide attempts.[288]

Depression can also impact physical health. Individuals with depression often have difficulty sustaining motivation to pursue beneficial health behaviors, such as exercise,[289] weight loss,[289] and compliance with treatment regimens for physical health conditions such as HIV,[290] diabetes,[291] and heart disease.[292] Depression can also lead to engagement with behaviors that are harmful to health including consumption of unhealthy food,[293] smoking,[294] and non-suicidal self-injury.[295] Depression is also associated with physiological changes that can adversely impact physical health, including alterations of stress hormone levels and responses[296] and suppression of the immune system.[297] Depression is associated with increased risk of mortality from numerous diseases including diabetes, cardiovascular disease, and stroke.[298] Depression is likely to play numerous roles in contributing to this increase in mortality, including contributing to

disease risk through biological[297] and behavioral[34] mechanisms, decreasing treatment-seeking for physical conditions,[299] decreasing adherence to treatment regimens,[291] reducing access to physical health care due to mental health stigma,[300,301] and reducing access to psychosocial support during physical illness.[302]

Like many mental health conditions, depression is often regarded as less severe or less legitimate than physical health issues.[303] However, as illustrated in this section, depression can have numerous and sometimes severe impacts on the lives of affected individuals. Trivialization or stigmatization of depression and other mental health issues can worsen their impact, and reduce affected individuals' ability to manage their disorder and its effects on their lives.[304,305] According to the World Health Organization, depression is now the leading cause of disability worldwide.[306] Recognition of depression as a serious concern equal to physical health disorders is essential for an informed and effective approach to public health.

Applying Findings from Genetic Epidemiology to Population Health

Studies using genetic data offer a unique opportunity to examine questions regarding the causal nature of relationships that are difficult to assess with traditional observational study designs. While findings from studies incorporating genetic data can inform novel pharmacological targets,[307,308] the intervention strategies that can be informed from the insights of genetic studies are not limited to biomedical treatments. For example, the results from genetic studies can offer guidance and justification for behavioral and environmental interventions geared towards both treatment and prevention. These can be interventions targeted towards individuals with high-risk genotypes, such as newborn screening for phenylketonuria,[309] or interventions based on biological knowledge gained from genetic data, such as exploring the effectiveness of inflammation-lowering lifestyle modifications among individuals with depression.[310]

Summary of Dissertation Findings

As shown in the preceding chapters, the relationships between inflammation, atopic disorders and depression are complex. Potential explanatory models include causal relationships

between the phenotypes, shared genetic or environment risk, unmeasured confounding, and differential measurement error. Moreover, these explanatory models are generally not mutually exclusive, and it is possible that multiple processes, at different points in the life course, are salient to the etiologic relationship between atopy and depression. While this dissertation focuses on this complexity as it relates to depression, inflammation, and atopy, these challenges are present in any effort to investigate the intersection of mental and physical health.

One of the core questions this thesis sought to address is: *Is there a causal relationship between inflammation and depression?* Applying a Mendelian Randomization design to a large sample of adults from the UK Biobank, the results from Chapter 3 support the hypothesis that inflammation (specifically, IL-6 signaling) has a causal effect on depression. Although this evidence indicates that inflammatory signaling has a causal effect on depressive symptoms in some individuals, not all individuals with depression have elevated systemic inflammation.[216] The importance of this distinction is demonstrated by a recent depression treatment clinical trial of the TNF-α inhibitor medication infliximab, which was found to improve in depressive symptoms only in individuals who had elevated levels of TNF-α prior to treatment.[48] The pervasive health effects of chronic elevations in systemic inflammation, which also include physical health disorders such as heart disease and diabetes,[311,312] underline the importance of clarifying the etiologic nature of the relationship between depression and inflammation. By adopting a more integrative approach to health, such work will help inform efforts to target inflammation as a risk factor for a wide range of medical and psychiatric conditions.

Another core issue this thesis sought to clarify is the relationship between atopic disorders (i.e., asthma, allergies) and depression, using both traditional observational epidemiologic methods and genetically-informed approaches. In contrast to the relatively straight-forward findings regarding IL-6 signaling, this thesis illustrates that the etiologic relationship between atopy and depression is more challenging and not yet fully resolved. Using data from the Collaborative Psychiatric Epidemiologic Surveys (CPES) a large and diverse sample of US adults, Chapter 2 reports the results of a cross-sectional analysis between allergic rhinitis and four of the most common mood and anxiety disorders in the general population. This analysis shows that several mental health disorders (including depression) have associations with atopy that are independent of psychiatric comorbidities.

Chapter 4 built on these results by applying genetically-informed methods to examine the possible causal nature of the relationship between depression and a range of atopic disorders using data from the UK Biobank. Again, applying the Mendelian Randomization design, the findings from Chapter 4 are not supportive of a causal relationship between atopy and depression. However, as noted above, this does not in and of itself exclude the possibility that such causal explanations may be relevant in at least some individuals.[248,313] In addition, the findings from Chapter 4 provide evidence consistent with shared genetic liability to depression and atopy, which replicates and extends prior work,[151,234,235] and has implications for both screening and clinical intervention. Taken together, the findings from Chapter 4 provide a more complete understanding of the salience of various explanatory models of the relationships between depression and atopy than had been explored in a single dataset previously.

It is worth noting that while there was not a significant association between depression and asthma in Chapter 2, preliminary analyses for Chapter 4 found a significant association between the two phenotypes. This difference between the two chapters is likely to result from differences in the datasets, sample sizes, and phenotype definitions used in the two chapters. Chapter 2 used the Collaborative Psychiatric Epidemiology Surveys (N=10,309, depression phenotyping based on the CIDI structured interview and DSM-IV criteria),[139,140] while Chapter 4 used data from UK Biobank (N=331,664, depression phenotyping based on two cardinal symptoms).[2] Additionally, in Chapter 2 the estimated odds ratios for the association between asthma and depression were all greater than 1.0, and might have been able to cross the threshold for statistical significance if the sample had been larger.

Future Directions for Research

The findings in Chapters 2-4 suggest several interesting areas for future research. One of the greatest limitations of Chapter 3 was the inability to be certain that any analysis (including use of summary statistics from a GWAS conditional on rs2228145 genotype) had fully removed the effects of the pleiotropic SNP rs2228145. A future study with access to individual-level sIL-6R data could stratify by rs2228145 genotype, to ensure that the effects of this SNP are fully removed. Another interesting area for future research would be to further examine the shared genetic liability between depression and atopy reported in Chapter 4, and attempt to identify

specific biological pathways involved in the shared liability by using methods such as stratified LD Score Regression which can partition heritability by SNP functional annotations.[314] Finally, although the polygenic risk score comparison in Chapter 4 was an interesting analysis to explore, the limited explanatory power of the polygenic risk scores used in that chapter (maximum R^2 = 1.8%) make it unlikely that such an analysis could succeed in meaningfully examining the questions it was intended to address. Future studies wishing to examine questions such as whether depression influences susceptibility to atopic responses may benefit from using other methods such as twin-based study designs.[7,315]

Informing an Integrative Approach to Mental and Physical Health

The findings from Chapter 3 are consistent with a causal effect of IL-6 signaling on depression, which suggests several avenues for interventions. These include applications such as the exploration of drugs which inhibit IL-6 signaling as depression treatments,[176,177] and the potential use of biomarkers on inflammation to identify which depression treatments are more likely to succeed in specific individuals based on their inflammatory phenotype.[316–318] Interventions that target factors that influence IL-6 signaling, whether environmental factors such as air quality or health behaviors such as tobacco use and diet quality,[99,181] would also have benefits to population health beyond individuals with diagnosed depression.[319]

These findings also suggest a need to take a more integrative approach to depression prevention, early identification, and clinical care, including incorporating and strengthening depression care management into routine medical care. This includes screening and monitoring individuals with medical conditions that are either characterized by or associated with systemic inflammation, such as autoimmune disorders[320] and obesity.[27] Additionally, regardless of whether the relationship between depression and atopy is causal, individuals with atopic disorders are at increased risk for depression[71] and may benefit from more frequent screening to identify early symptoms and reduce the barriers to depression treatment.

Addressing shared risk factors contributing to multiple diseases can be particularly important targets for public health interventions, because modification of these risk factors can lead to simultaneous improvement in numerous health outcomes. As one example, individuals who experienced childhood poverty are at increased risk for obesity, systemic inflammation,

depression, heart disease, and many types of cancer.[321] While a medication that inhibits inflammatory signaling may have the potential to improve depressive symptoms among those who receive it, an intervention targeting childhood poverty might protect many individuals from depression and numerous other negative health outcomes. The existence of interventions at one level does not eliminate the need for interventions at other levels, and a multi-level approach is essential for addressing depression and numerous other aspects of public health.

Conclusions

By leveraging the tools of genetic epidemiology to address questions regarding the causes of depression and its relationships to conditions like atopy, we can better understand this complex and important health condition. These efforts can contribute to the development of interventions that seek to prevent and/or address the harmful effects of this disorder for individuals, their families, and on population health as a whole. Depression is thought to be influenced by factors at every level, from the genetic to the social, and can have wide-ranging impacts on all aspects of life. As the world's leading cause of disability, depression substantially effects mental, physical, social, and economic wellbeing.[298,322,323] Because of this, as leaders in the field have noted, there truly can be "no health without mental health."[324]

Appendices

Appendix A: Supplemental Note for Chapter 3

Preparation of UK Biobank data

Depression phenotyping

The primary phenotype definition for the UK Biobank sample was "recurrent depressive symptoms", defined as reporting a lifetime history of at least one of the cardinal symptoms of depression (low mood or anhedonia)[2] occurring on at least two occasions. The early versions of the UK Biobank intake interview used in 2006-2008 did not include questions about lifetime depression history or recurrence,[260] so information from three questionnaires (the updated intake interview used in 2009-2010, the first repeat visit in 2012-2013, and the online mental health supplement introduced in 2016) was combined to create the largest possible sample size. For individuals who had completed more than one depression questionnaire, we used their most recent questionnaire to assess their depression phenotype. We excluded individuals whose lifetime history of depression was inconsistent from classification as controls (eg. those who reported no lifetime history of depressive symptoms on their most recent depression questionnaire, but had previously reported depressive symptoms in an earlier questionnaire.)

A second more stringent phenotype of "recurrent DSM-V major depression" was defined as reporting at least 5 depressive symptoms on at least two occasions.[2] Because this phenotype could only be assessed in individuals who completed the more detailed online supplement, it resulted in a smaller sample size (n=52,055), and was therefore only used as a secondary analysis, without exclusion of potential overlap with the PGC MDD 2018 sample, to produce a sample size of 74,563.

Both phenotype definitions excluded individuals with an ambiguous depressive phenotype (i.e., those who had experienced some depressive symptoms but did not meet the full case definition including recurrence).

Eligibility criteria

Eligibility criteria included availability of data about cardinal symptoms of depression and depression recurrence from either the UK Biobank intake assessment, repeat visit, or OMH questionnaires, and availability of genetic data that passed quality control checks. To minimize overlap with the PGC MDD 2018 sample, individuals who were potentially included in PGC MDD 2018's UK Biobank sample (defined as "included in the UK Biobank pilot genetic data release" and "had complete data for depression phenotyping") were excluded from the UK Biobank analysis. Exclusion criteria for other mental illness consisted of a self-reported history of schizophrenia or bipolar disorder, reporting a potential period of mania severe enough to be life-disrupting or require treatment, reporting use of medication to treat psychotic symptoms, or classification as "probable bipolar" under the phenotypes defined in Smith et al 2013.[260]

Due to a high degree of cryptic relatedness in the UK Biobank sample,[222] PRIMUS (version 1.9.0)[325] was used to identify which individuals to exclude to produce the "maximum unrelated subset", with depression cases preferentially preserved. The threshold used to define relatedness was third degree or closer relation, defined using a kinship coefficient cutoff value of $2^{-9/2}$ (0.04419).[326] The depression phenotype definitions are illustrated in Figures C.2 and C.4, and full details of inclusion and exclusion criteria are shown in a STROBE diagrams in Figures C.1 and C.3.

Genetic quality control and GWAS analyses

This analysis used imputed genetic data released by UK Biobank, which had already been through several genetic quality control steps.[222] Additional quality control steps were performed prior to conducting the analysis, including repetition of Hardy-Weinberg equilibrium testing to address possible issues reported by another study using UK Biobank data,[327] and filtering of SNPs to restrict analysis to those with a minor allele frequency of at least 1%, a missingness rate of no more than 5%, and an imputation INFO score of at least 0.7. Prior to Mendelian Randomization analysis, genetic data was also filtered to restrict analyzed variants to single

nucleotide polymorphisms (SNPs), and to use only biallelic SNPs. Only alleles with a frequency of at least 1% were considered when assessing whether each SNP qualified as bilallelic.

We conducted GWAS analyses for the depression phenotypes using Plink 2.0 alpha. All GWAS included sex, age at the time of the UK Biobank intake interview, and 20 genetic principal components provided by UK Biobank with the genetic data release. For each GWAS, we examined QQ plots to check for signs of inflation or other abnormalities.

Selection of independent SNPs for the Maximum Likelihood method

For the van Dongen 2014 sIL-6R coefficients and the Framingham sgp130 coefficients, we performed SNP selection based on LD clumping using Plink 1.9 with r^2 threshold of 0.001 and 0.01 over a distance of 10,000 kilobases. The results from these studies included only SNPs which had reached genome-wide significance, and therefore could not be processed using GCTA-COJO, which requires full GWAS results to calculate phenotypic variance.[328]

For the IMPROVE coefficients, we also used the conditional joint analysis (COJO) feature of GCTA[329] 1.92.2 beta was used to select SNPs that were significant in the original analysis and remained significant in the conditional analysis at p-value cutoffs of $5*10^{-8}$, $1*10^{-6}$, and 0.0001. When GCTA-COJO was used to perform SNP selection, COJO-adjusted betas and standard errors for SNPs were used in the subsequent Mendelian Randomization analyses.

For both SNP selection methods, we ensured inclusion of rs2228145 (or its best-available proxy) by excluding other SNPs in LD with rs2228145 ($r^2 \geq$ the clumping threshold , or $r^2 \geq 0.5$ for GTCA-COJO) from the SNP selection process. We did this so that the effect of rs2228145 would be captured by a single SNP and its effects could easily be examined in forest plots and leave-one-SNP-out plots.

Mendelian Randomization considerations and requirements

The three assumptions required for Mendelian Randomization

In order for Mendelian Randomization to be an a valid instrumental variable analysis suitable for use in causal inference, three requirements should be met:[100,195,273,330]

Assumption 1: There must be an association between the genetic variant(s) and the hypothesized causal exposure.[100,195,273,330]

This requirement could be fulfilled by most SNPs identified in GWAS of the exposure, however knowledge of the biological mechanism through which the genetic variant influences in the exposure is recommended to avoid unintended consequences from indirect effects.[100] In the current Mendelian Randomization analysis, this requirement is addresses by using only SNPs having a significant association with sIL-6R in a genome wide association study. All SNPs in the analysis are located on chromosome 1 near the IL6R gene, making a direct biological effect plausible, and the strongest SNP (rs2228145) is a missense variant that increases sIL-6R levels via a known biological mechanism.[189]

Assumption 2: The genetic variant(s) must not influence the outcome in any way other than through influence on the exposure.[100,195,273,330]

This requirement can be violated by genetic variants with pleotropic effects.[100,195,273,330] Pleiotropy can be divided into two categories, which can have different impacts on Mendelian Randomization.[100] In vertical pleiotropy, one phenotype is influenced as a downstream consequence of the other, such as LDL cholesterol and heart disease.[100,331,332] Vertical pleiotropy has been described as "the very essence" of Mendelian randomization, and does not violate this assumption.[100] Horizontal pleiotropy occurs when multiple phenotypes are associated with a genetic variant for reasons other than a cause-and-effect relationship between the phenotypes, and is a violation of Mendelian Randomization assumptions.[100] We used multiple methods to check for horizontal pleiotropy during analyses, including MR Egger regression for the SNPs used with the Maximum Likelihood method,[333] and HEIDI outlier analysis used with the GSMR method.[198]

The effects of rs2228145 on both IL-6 receptor types could also be considered a violation of the pleiotropy assumption if the analysis were used to make inferences specific to one of the IL-6 receptors. To address this consideration, we performed additional analyses to examine which receptor type was responsible for the relationship between rs2228145 and depression, and to examine the consistency of results when using SNPs filtered to exclude those in partial LD

with rs2228145. Because these additional analyses did not produce strong and conclusive results, the main conclusions from this paper are limited to reporting a causal effect of IL-6 signaling on depression without specification of which IL-6 receptor type is involved in the causal pathway.

Assumption 3: The genetic variant(s) must not be associated with potential confounders of the relationship between the exposure and the outcome.[100,195,273,330]

This requirement can be violated when the genetic instrumental variable is correlated with an environmental factor in the study population. The primary mechanism through which this assumption can be violated is population stratification. In population stratification, sub-groups within the population have different ancestral backgrounds and therefore potentially different allele frequencies.[334] If these sub-groups experience different levels of exposure to environmental risk factors, such as stress, these exposures can create the appearance of an association between the genetic instrumental variable and the outcome. To meet this Mendelian Randomization requirement, this study used only individuals of European ancestry, and also used genetic principal components during GWAS analysis to account for the possibility of subtle stratification present in the UK population.[335,336]

Weak instrument bias

Weak instrument bias can occur when the genetic instrumental variable explains only a small proportion of the variance in the exposure variable,[337] which can allow subtle correlations or confounding to bias analysis results.[338] Weak instrument bias is best avoided by choosing an instrument that has a strong association with the exposure, a requirement met by rs2228145 which explains 51% of the variance in sIL-6R levels.[189] When no single genetic instrument explains a large proportion of the variance in the exposure, multiple variants may be used in combination,[339,340] an approach also applied in several analyses in this paper. Finally, all analyses in this paper use a two-sample Mendelian Randomization design, so any effects from weak instrument bias would be in the direction of the null hypothesis.[273]

Sample overlap

Two-sample Mendelian Randomization analyses can be biased by the inclusion of some individuals in both samples (sample overlap).[250] To address this possibility, this analysis attempted to use samples originating from different countries, such as using the UK Biobank Sample (UK) with the van Dongen 2014 sample (Netherlands). In some cases where this approach was not feasible (such as with the PGC MDD 2018 coefficients, which come from a meta-analysis of cohorts in several countries) the large international nature of the samples means that any overlap would be limited to a small percent of the sample.

A notable amount of sample overlap is likely when the van Dongen 2014 coefficients are analyzed in combination with the PGC MDD 2018 coefficients, as both studies included participants from the NESDA cohort. However, the current study used multiple combinations of exposure and outcome samples, to ensure that results replicated across combinations and could not result from an overlap specific to one set of exposure and outcome samples.

Diagnostic test results

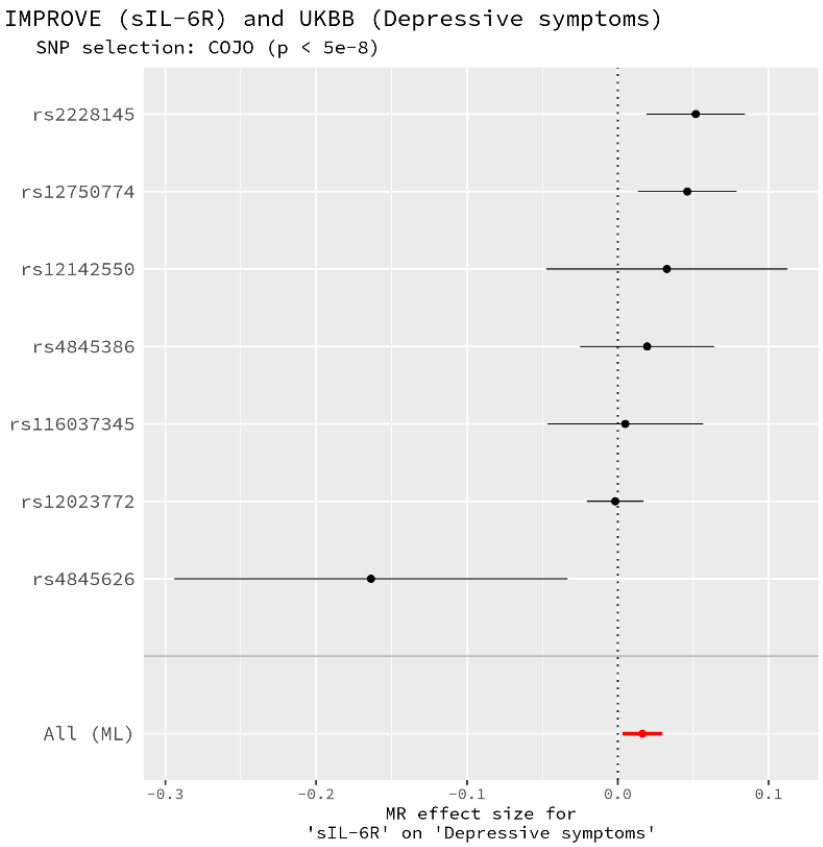
Three diagnostic statistical tests were used to assess compliance with Mendelian Randomization requirements. Although statistical testing on its own is not sufficient to detect all possible assumptions of the Mendelian Randomization requirements, such tests can be useful to flag specific problems.

For GSMR analyses, the HEIDI outlier test detected and excluded two SNPs when examining the IMPROVE sIL-6R coefficients in combination with the PGC MDD 2018 coefficients at r^2 clumping thresholds 0.05 (rs12739228), 0.1 (rs12739228), and 0.2 (rs192423521).

For analyses using selected sets of independent SNPs, the MR-Egger intercept was not significant for any analysis. We also tested for heterogeneity across instruments using Cochran's Q test and found significant heterogeneity when using IMPROVE coefficients in combination with UK Biobank coefficients and SNP sets produced by GCTA-COJO SNP selection (all three p-value thresholds). Visual inspection of forest plots for these analyses indicated that the heterogeneity was largely driven by rs4845626, which produced notably lower estimates than other SNPs selected in the analyses, as illustrated below. Because the direction of any bias

introduced by a low outlier would be towards the null hypothesis, we retained this SNP in the analysis.

Figure A.1: Forest plot showing r4845626 as an outlier in the IMPROVE/UKBB analysis



Appendix B: Supplemental Note for Chapter 4

Eligibility criteria

Eligible participants were UK Biobank participants of white British ancestry who had genetic data and passed genetic quality control. Exclusion criteria for other mental illness consisted of a self-reported history of schizophrenia or bipolar disorder, reporting a potential period of mania severe enough to be life-disrupting or require treatment, or reporting use of medication to treat psychotic symptoms.

Due to a high degree of cryptic relatedness in the UK Biobank sample,[222] PRIMUS (version 1.9.0)[325] was used to identify which individuals to exclude to produce the "maximum unrelated subset". The threshold used to define relatedness was third degree or closer relation, defined using a kinship coefficient cutoff value of $2^{-9/2}$ (0.04419).[326]

Recurrent depressive symptoms phenotype

Because questions about lifetime history of depression were only included during the final two years of the UK Biobank intake period,[260] we combined data from three depression questionnaires included in the UK Biobank data to produce a larger sample size. We defined the phenotype "recurrent depressive symptoms" as experiencing at least one cardinal symptom of depression (low mood or anhedonia)[2] on at least two occasions. We classified participants as controls if they did not report any periods during which they experienced cardinal symptoms of depression. We assessed this phenotype using data from the UK Biobank intake interview, the UK Biobank first repeat visit, and the Online Mental Health Supplement.

In cases where individuals had completed more than one depression questionnaire, we determined their status using the most recent questionnaire they had completed. To ensure that controls did not have a lifetime history of depression, we classified participants as controls if

they met control criteria at their most recent assessment, and did not have any other status (case, ambiguous phenotype, or a "don't know/refused" answer) on any earlier depression questionnaires that they had been asked. Because an individual can transition to case status at any time, we did not perform a similar evaluation of past responses for individuals who qualified as cases at their latest assessment.

While the UK Biobank intake and repeat visit sessions used the same touchscreen questions, these questions differed from the ones found in the online mental health questionnaire. The touchscreen questions asked whether an individual had experienced each cardinal symptom for "a whole week", while the online questions asked whether an individual had experienced an episode of symptoms "for two weeks or more in a row". We examined the possibility of adding the touchscreen questions regarding symptom duration to the phenotype definition to enforce a two-week minimum ("How many weeks was the longest period when you were feeling [insert symptom]?"), however we found that these duration questions had missing data for approximately 18% of potential cases. Due to the high missingness, and the fact that these questions applied only to the longest episode and not all episodes, we decided not to include duration in the phenotype definition.

Selection of samples for analyses

Many of the analyses used in this study are sensitive to overlap between samples. To address the particular requirements of each analysis, we enforced different sample selection criteria.

To create non-overlapping samples, any participant who had been asked one or more sets of questions about lifetime depression history was allocated to the *Complete Depression Data* sample. Participants who had never been asked about lifetime history of depression (eg. participants who received a version of the intake interview that did not include the necessary questions, were not included in the first UK Biobank repeat visit, and did not participate in the online mental health questionnaire) were allocated to the *Atopic Phenotypes Only* sample. To avoid selection bias, individuals who had participated in an interview including depression questions but had skipped the questions or who had an ambiguous depressive phenotype were

not included in the atopic disorders sample. These non-overlapping samples were used for the Mendelian Randomization analyses and the Polygenic Risk Score analysis.

Because LD Score Regression is not sensitive to sample overlap, for this analysis we used the same phenotype definitions in all eligible individuals, without creating separate non-overlapping samples.

The PGC MDD 2018 sample included 29,740 individuals from a pilot release of the UK Biobank data. In order to maximize sample size in the UK Biobank depression sample, we did not exclude potential overlap with the PGC MDD 2018 sample, as the only analysis in which the two phenotypes were analyzed together (LD Score Regression) was not sensitive to sample overlap. No overlap was possible between the non-overlapping atopic disorders sample (used for Mendelian Randomization) and the PGC MDD 2018 sample because any individuals with the necessary data to quality for the PGC MDD 2018 sample would have been included in the UKB depression sample rather than in the *Atopic Phenotypes Only* samples.

Genetic quality control

This analysis used imputed genetic data released by UK Biobank, which had already been through several genetic quality control steps.[222] Additional quality control steps were performed prior to conducting the analysis, including repetition of Hardy-Weinberg equilibrium testing to address possible issues reported by another study using UK Biobank data,[327] and filtering of SNPs to restrict analysis to those with a minor allele frequency of at least 1%, a missingness rate of no more than 5%, and an imputation INFO score of at least 0.7. Prior to Mendelian Randomization analysis, genetic data was also filtered to restrict analyzed variants to single nucleotide polymorphisms (SNPs), and to use only biallelic SNPs. Only alleles with a frequency of at least 1% were considered when assessing whether each SNP qualified as bilallelic.

GWAS

We conducted GWAS analyses for the depression phenotypes using Plink 2.0 alpha. All GWAS included sex, age at the time of the interview used to ascertain the phenotype under study, and 20 genetic principal components provided by UK Biobank with the genetic data

release. For each GWAS, we examined QQ plots to check for signs of inflation or other abnormalities. In cases where phenotypes were used with different sub-samples in different analyses, multiple GWAS were conducted for each phenotype within the appropriate sample, eg. GWAS for the Allergy/Eczema phenotype were conducted using the full UK Biobank sample, the *Atopic Phenotypes Only* sample, and in additional sub-samples required for the PRS analysis.

Appendix C: Supplemental Tables and Figures for Chapter 3

Table C.1: Samples used in the analysis

Sample	Phenotype	Unit of measurement	N	Age range	% Female	Covariates used in model*	Source Countries
van Dongen 2014[189]	sIL-6R blood levels	pg/mL (original) $1*10^{-8}$ g/mL (converted)	4846	18-90	61.3%	Unknown	Netherlands
IMPROVE[193]	sIL-6R blood levels	log(2) log(10) pg/mL	3394	55-79	Unknown, both sexes included	age, sex, smoking, diabetes, hypertension	Finland, France, Italy, The Netherlands, Sweden
UK Biobank[222]	Recurrent depressive symptoms	Case/control, see Figure C.2 or supplementary note for phenotype definition	89,119	40-80	53.1%	Age, sex	United Kingdom
UK Biobank[222]	Recurrent DSM-V major depression	Case/control, see Figure C.4 or supplementary note for phenotype definition	74,716**	46-80	53.3%	Age, sex	United Kingdom

PGC MDD 2018[194]	Major Depressive Disorder	Case/control using definitions from DSM-IV, ICD-9, or ICD-10	173005	Adults	Unknown, both sexes included	Meta-analysis, see original paper for further details	Germany, Australia, Switzerland, United Kingdom, United States, Netherlands, Ireland, Denmark, Sweden
KORA[205]	sgp130 blood levels	Relative fluorescence	997	32-81	Unknown, both sexes included	Age, sex, body mass index	Germany
Framingham[206]	sgp130 blood levels	Unknown	5257	Adults	53%	Age, sex, body mass index	United States
GTEx[207]	IL6R gene expression (blood)	Normalized gene expression	838	21-70	Unknown, both sexes included	Sex	United States
Westra 2013[209]	IL6R gene expression (blood)	Normalized gene expression	5311	Adults	Unknown, both sexes included	Meta-analysis, see original paper for further details	Estonia, Finland, Germany, Italy, Netherlands, United Kingdom, United States
CAGE[208]	IL6R gene expression (blood)	Normalized gene expression	2765	Adults	Unknown, both sexes included	Meta-analysis, see original paper for further details	Australia, Estonia, Morocco, United States

* Column does not show genetic principle components, or technical or study-specific factors such as batch, cell counts, or assessment center.

** This phenotype was used as a supplementary analysis only. Because fewer people had data available for this phenotype, individuals who potentially overlapped the PGC MDD 2018 sample were *not* excluded in this sample (unlike for the phenotype "recurrent depressive symptoms″ which excluded potential overlap with the PGC sample.)

Table C.2: Descriptive characteristics of UK Biobank sample "Recurrent Depressive Symptoms" phenotype

	No History of Depression (n=56,047)	History of Depression (n=33,072)
Age (mean, SD)	62.2 (8.5)	60.3 (8.4)
Age (range)	40-80	40-79
Female	26090 (46.6%)	21211 (64.1%)
College or university degree	20152 (36.2%)	12796 (38.9%)
rs2228145 genotype		
A/A	19700 (35.1%)	11368 (34.4%)
A/C	27031 (48.2%)	15969 (48.3%)
C/C	9316 (16.6%)	5735 (17.3%)

Table C.3: Descriptive characteristics of UK Biobank sample "Recurrent DSM-V Major Depression" phenotype

	No history of depression (n=53,368)	History of Depression (n=21,195)
Age (mean, SD)	65.0 (7.6)	62.3 (7.5)
Age (range)	46 - 80	46 - 79
Female	24566 (46%)	15228 (71.8%)
College or university degree	9413 (44.6%)	9413 (44.6%)
rs2228145 genotype		
A/A	18760 (35.2%)	7348 (34.7%)
A/C	25658 (48.1%)	10186 (48.1%)
C/C	8950 (16.8%)	3661 (17.3%)

Table C.4: Mendelian Randomizations using sIL-6R and the "Recurrent DSM-V Major Depression" phenotype in UK Biobank data

		UK Biobank Sample		
	Method / SNP selection	Odds ratio (95% CI)	P	# SNPs
van Dongen 2014 coefficients[a]	Ratio of Coefficients (rs12126142)	1.018 (0.998-1.037)	0.076	1
	Maximum Likelihood			
	Clumping at r2=0.001	**	**	2
	Clumping at r2=0.01	1.018 (0.999-1.037)	0.069	4
	GSMR			
	Clumping at r2=0.05	1.019 (1.000-1.039)	0.045	14
	Clumping at r2=0.10	1.017 (0.999-1.036)	0.068	23
	Clumping at r2=0.15	1.017 (0.999-1.036)	0.063	26
	Clumping at r2=0.20	1.017 (0.999-1.036)	0.070	28
	PCA-IVW	1.015 (0.996-1.035)	0.116	491 (4 PCs)
	PCA-IVW (conditional)*	1.011 (0.970-1.053)	0.601	275 (2 PCs)
IMPROVE coefficients[b]	Ratio of Coefficients (rs2228145)	1.026 (0.996-1.056)	0.092	1
	Maximum Likelihood			
	Clumping at r2=0.001	**	**	2
	Clumping at r2=0.01	1.028 (1.001-1.056)	0.044	4
	COJO at p=5e-8	1.013 (0.997-1.029)	0.106	7
	COJO at p=1e-6	1.014 (0.998-1.030)	0.085	9
	COJO at p=0.0001	1.013 (1.000-1.026)	0.042	14
	GSMR			
	Clumping at r2=0.05	1.020 (0.999-1.040)	0.061	11
	Clumping at r2=0.10	1.022 (1.001-1.042)	0.035	15
	Clumping at r2=0.15	1.016 (0.997-1.037)	0.106	22
	Clumping at r2=0.20	1.018 (0.998-1.037)	0.072	25
	PCA-IVW	1.012 (0.988-1.038)	0.323	517 (7 PCs)

[a] sIL-6R in units of $1*10^{-8}$ g/mL [b] sIL-6R in units of log pg/mL

* This analysis used coefficients from van Dongen 2014 supplementary table 3, a GWAS of sIL-6R conditional on rs2228145 genotype

** Not enough SNPs to perform analysis

Table C.5: Results of sgp130 Mendelian Randomizations

		UK Biobank Sample			PGC MDD 2018 coefficients		
Exposure coefficients	**Method**	**Odds ratio (95% CI)**	**P**	**# SNPs**	**Odds ratio (95% CI)**	**P**	**# SNPs**
Framingham[a]	Maximum Likelihood	1.011 (0.958-1.067)	0.689	8	1.010 (0.967-1.054)	0.659	8
Framingham[a]	PCA-IVW	1.020 (0.954-1.090)	0.562	201 (7 PCs)	1.010 (0.959-1.063)	0.709	207 (8 PCs)
KORA[b]*	PCA-IVW	1.003 (0.948-1.061)	0.919	3 (1 PC)	1.014 (0.962-1.068)	0.607	3 (1 PC)

[a] sgp130 measured in pg/mL [b] sgp130 measured in units of relative florescence

* Due to the small number of significant SNPs in the KORA results, only 2 SNP remained after clumping for LD at $r^2 = 0.001$ or 0.01, which prevented the use of Maximum Likelihood analysis.

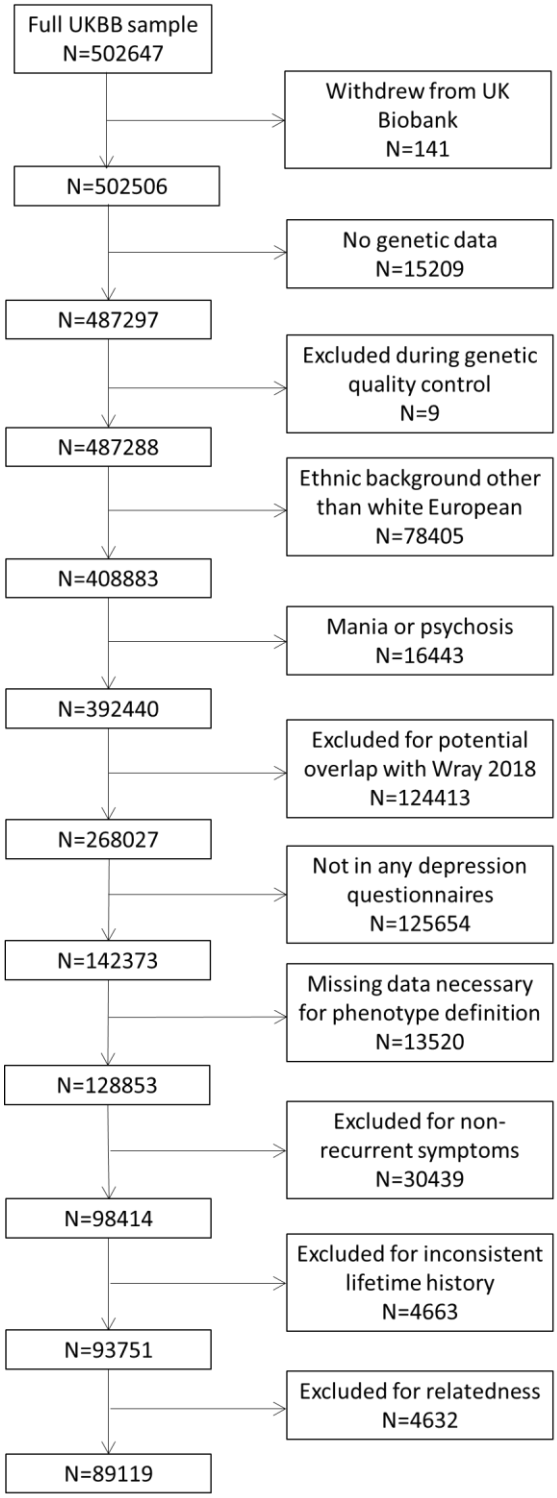
Table C.6: Results of Mendelian Randomizations for IL6R expression using eQTL data

	UK Biobank Sample			PGC MDD 2018 coefficients		
eQTL coefficients[a]	Odds ratio (95% CI)	P	# SNPs	Odds ratio (95% CI)	P	# SNPs
Westra	0.878 (0.801-0.964)	0.006	45 (3 PCs)	0.927 (0.861-0.998)	0.043	45 (3 PCs)
GTEx	0.874 (0.734-1.040)	0.128	111 (2 PCs)	0.909 (0.793-1.043)	0.175	116 (3 PCs)
CAGE	0.879 (0.805-0.960)	0.004	97 (3 PCs)	0.929 (0.866-0.996)	0.040	101 (3 PCs)

[a] All coefficients are in units of normalized gene expression

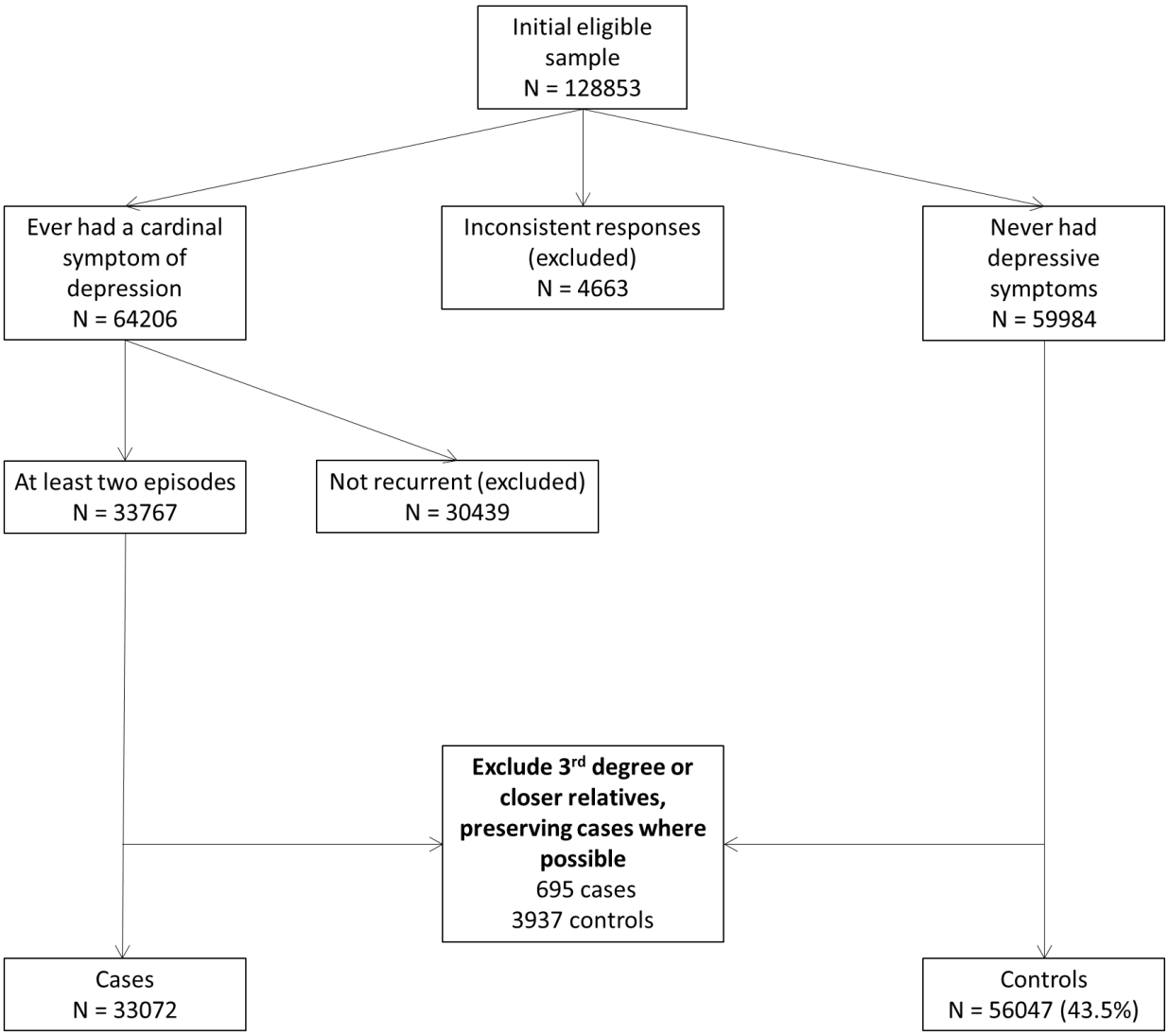
Table C.6 shows a tendency for increased expression of the IL6R gene to be associated with lower odds of depression, in contrast with the Table 3.2 result that higher circulating sIL-6R was associated with higher odds of depression. Due to linkage disequilibrium between eQTLs, all analyses use the PCA-IVW method. In cases where eQTL datasets contained information for more than one IL6R expression probe, the probe with the largest number of significantly associated SNPs was used for the analysis.

Figure C.1: STROBE diagram for UK Biobank sample "Recurrent Depressive Symptoms" phenotype



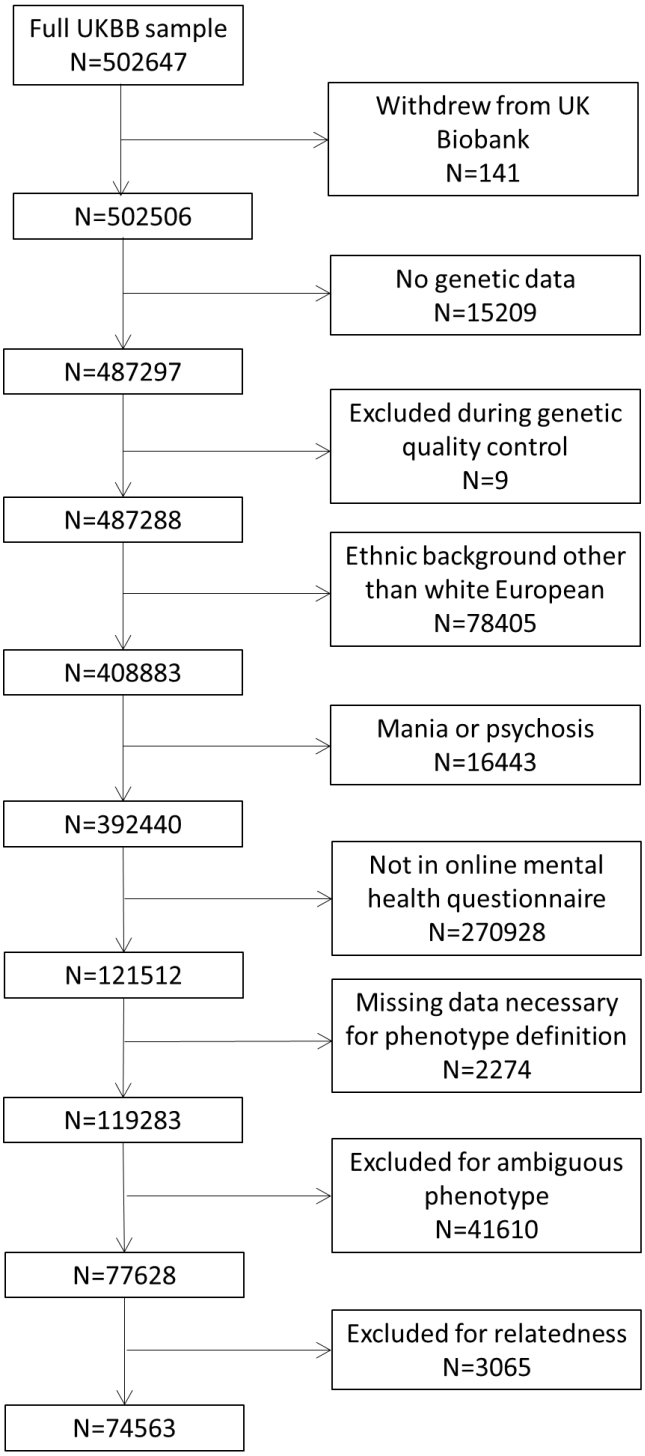
Note: The box "inconsistent lifetime history" refers to individuals who reported no lifetime history of depressive symptoms in their most recent depression questionnaire but had reported depressive symptoms in an earlier depression questionnaire. Further details regarding eligibility criteria are included in the supplemental note.

Figure C.2: Phenotyping flowchart for the "Recurrent Depressive Symptoms" phenotype



Note: The box "inconsistent responses" refers to individuals who reported no lifetime history of depressive symptoms in their most recent depression questionnaire but had reported depressive symptoms in an earlier depression questionnaire. Further details regarding eligibility criteria are included in the supplemental note.

Figure C.3: STROBE diagram for UK Biobank sample "Recurrent DSM-V Major Depression" phenotype



Note: Because this phenotype is used only in supplementary analyses and is not the primary analysis phenotype, we did not exclude potential overlap with the PGC MDD 2018 sample. With that exclusion, sample size for this phenotype would be reduced to 52,055.

Figure C.4: Phenotyping for the "Recurrent DSM-V Major Depression" phenotype

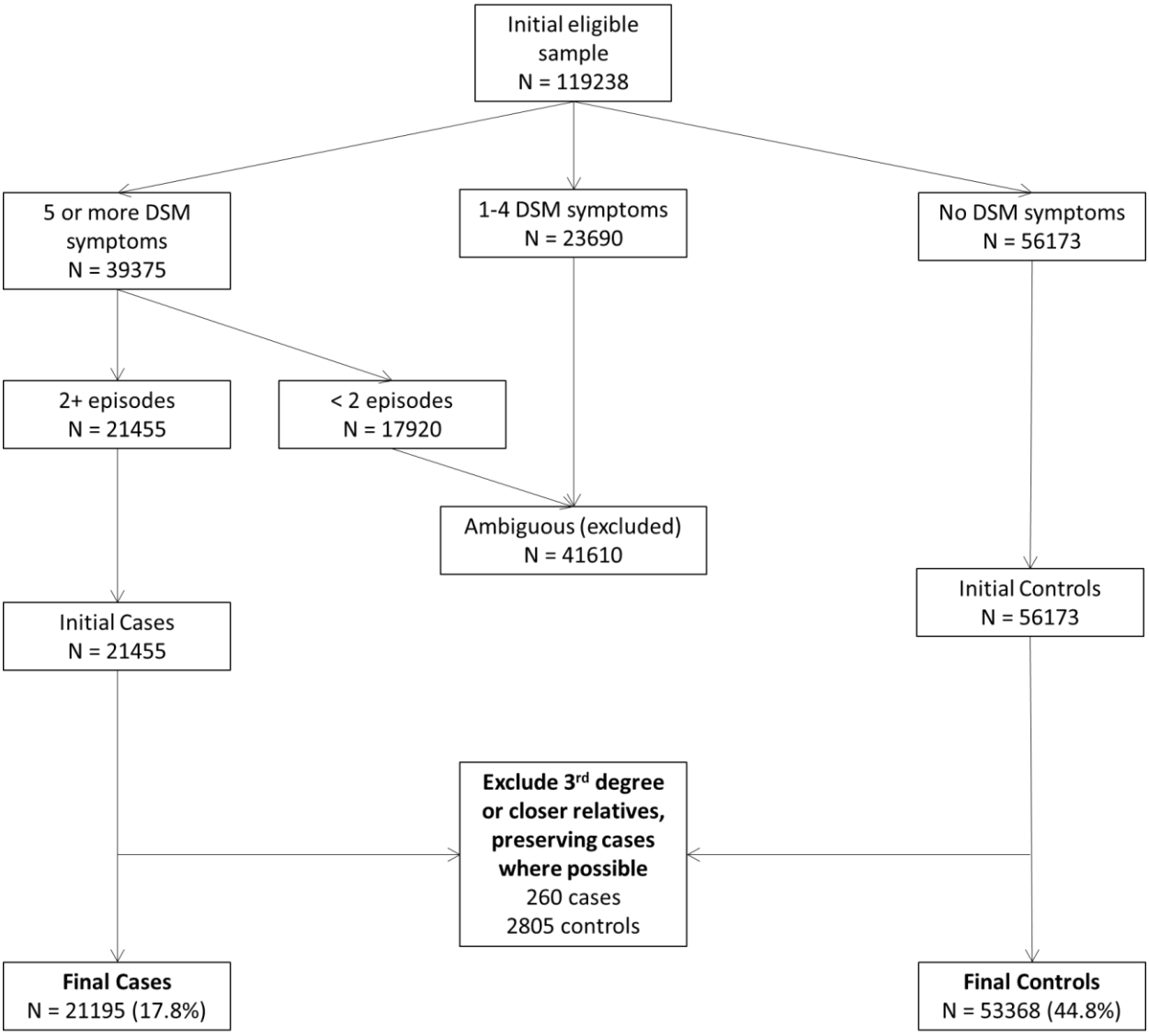
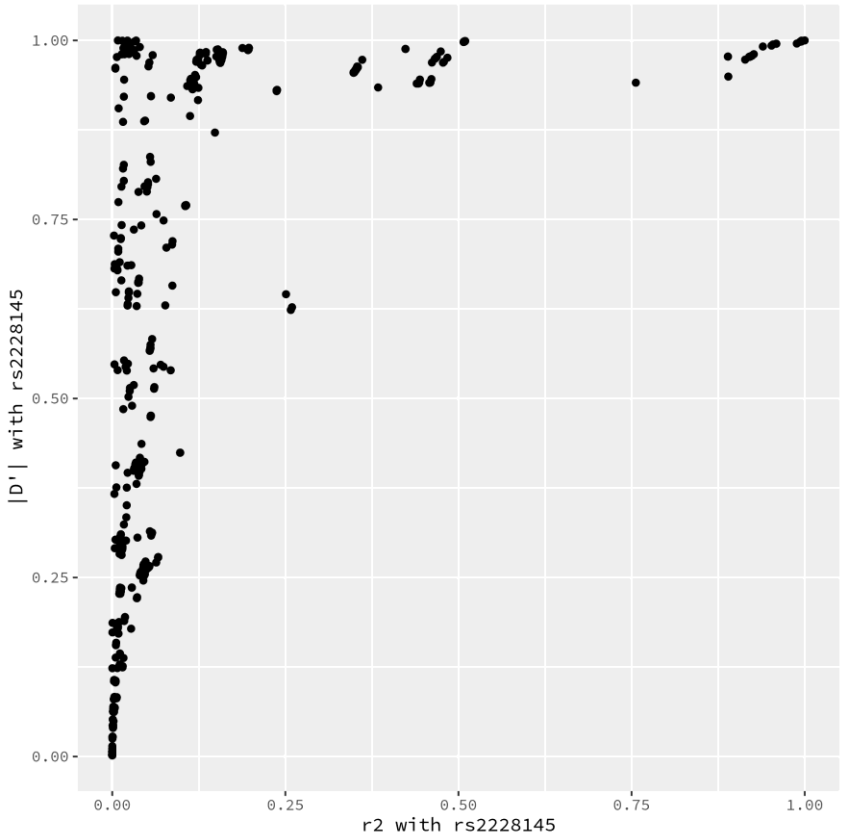


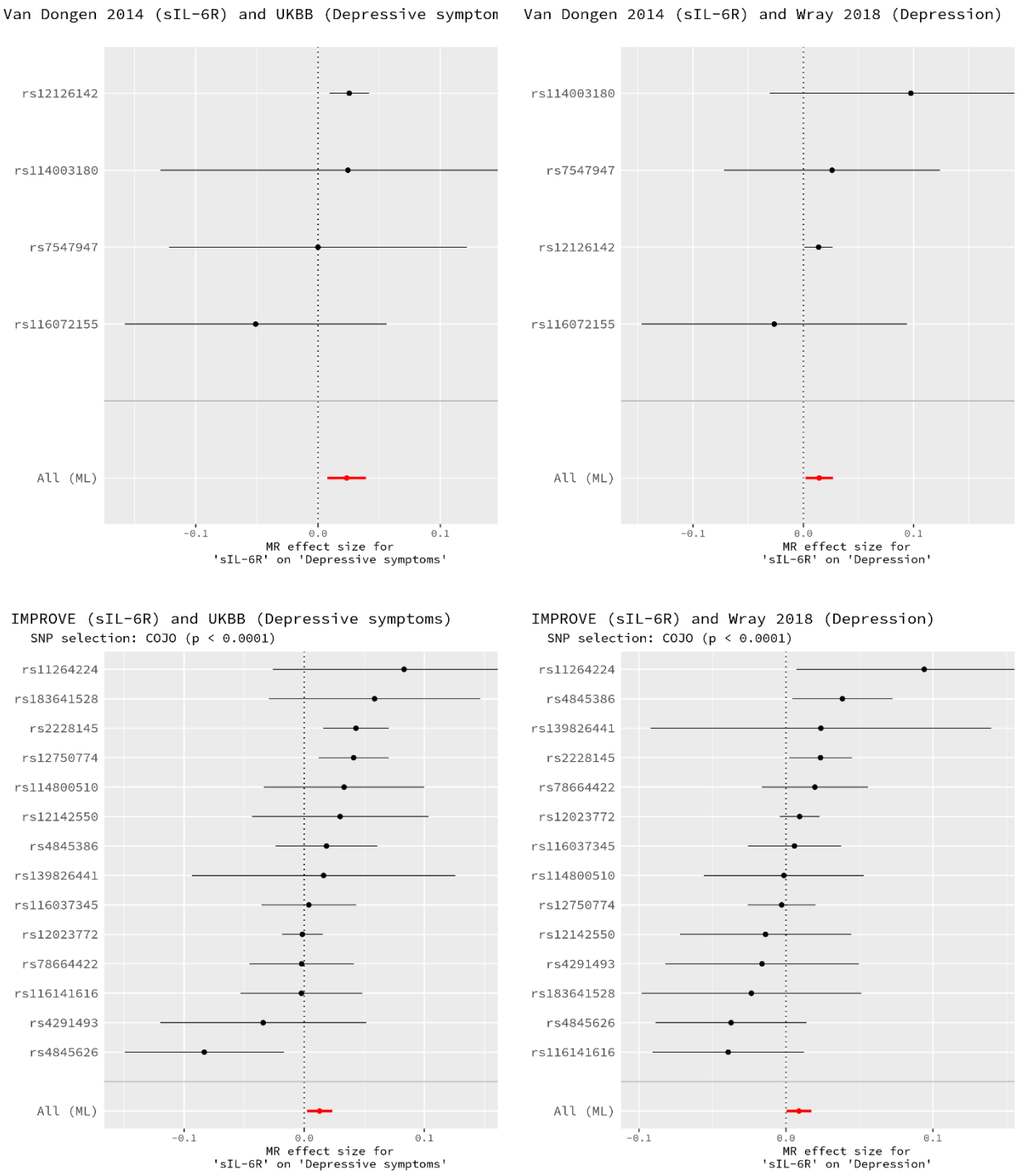
Figure C.5: Comparison of SNP |D'| and R^2 values with rs2228145



This plot shows SNPs found to be associated with sIL-6R in at least one exposure dataset (Van Dongen 2014 and IMPROVE), and compares the r^2 and Lewontin's |D'| statistics for each SNP's association with rs2228145.

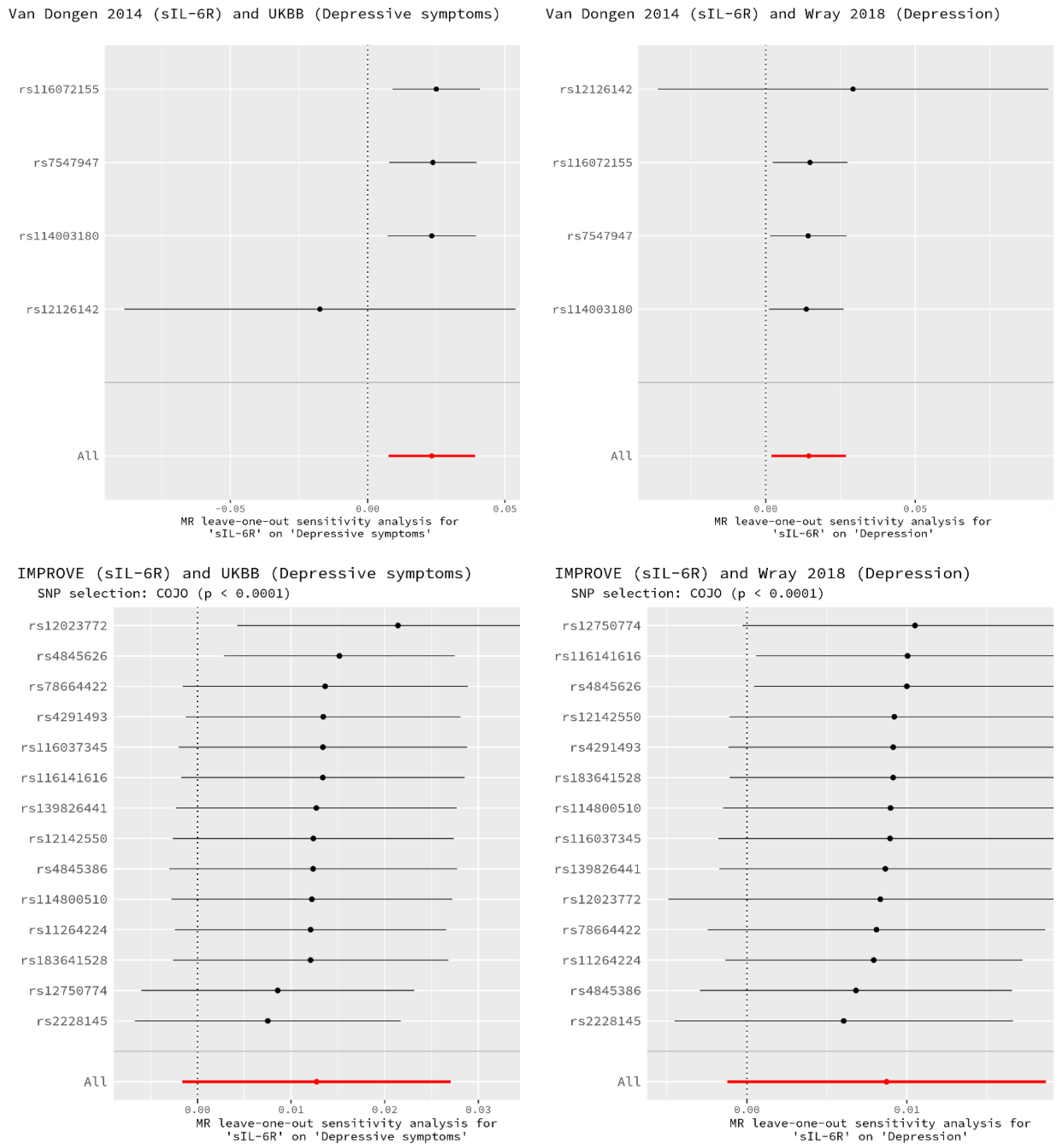
The presence of SNPs with low r^2 values with rs2228145 but moderate to high |D'| values demonstrates that clumping or pruning based on r^2 may not be selecting SNPs that are fully independent from rs2228145. Both LD measures were calculated using individual-level genetic data from European-ancestry UK Biobank participants.

Figure C.6: Forest plots for SNPs used in Maximum Likelihood analyses



For each combination of datasets, only the SNP selection method producing the largest number of SNPs is shown in the figures above. When GCTA-COJO was used for SNP selection, COJO-adjusted SNP betas and standard errors were used for the analysis.

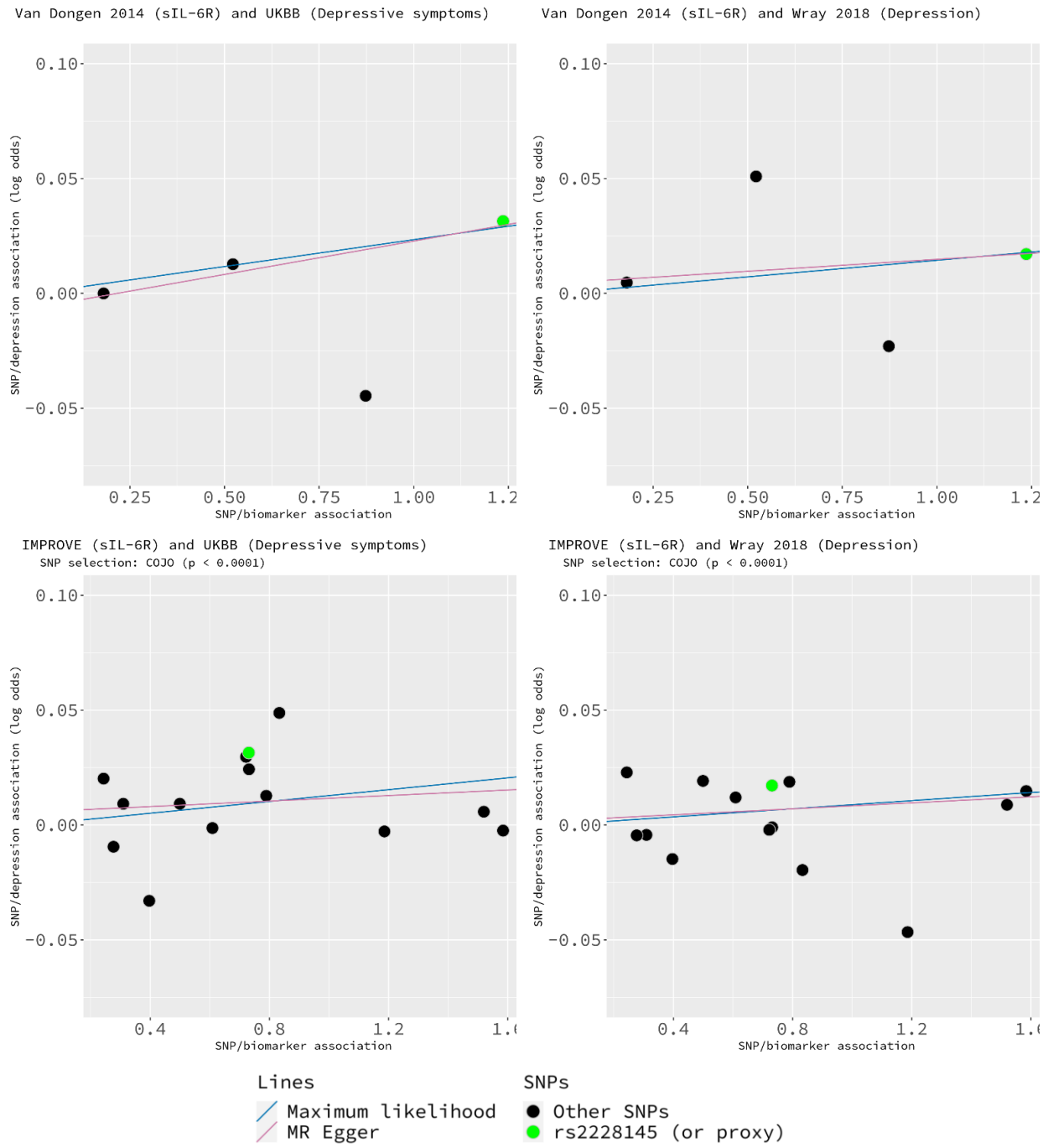
Figure C.7: Leave-one-SNP-out plots for SNPs used in Maximum Likelihood analyses



For each combination of datasets, only the SNP selection method producing the largest number of SNPs is shown in the figures above. When GCTA-COJO was used for SNP selection, COJO-adjusted SNP betas and standard errors were used for the analysis.

In all leave-one-out plots, omission of rs2228145 (or its proxy, rs12126142) resulted in the results losing significance. This is expected, because rs2228145 is a very strong genetic instrument, and loss of the strongest genetic instrument will reduce statistical power. Although the results without rs2228145 lose significance, in most cases the effect estimates remain positive, suggesting that the loss of significance without rs2228145 may be due to rs2228145's strength, and not due to rs2228145 having a different effect than other SNPs.

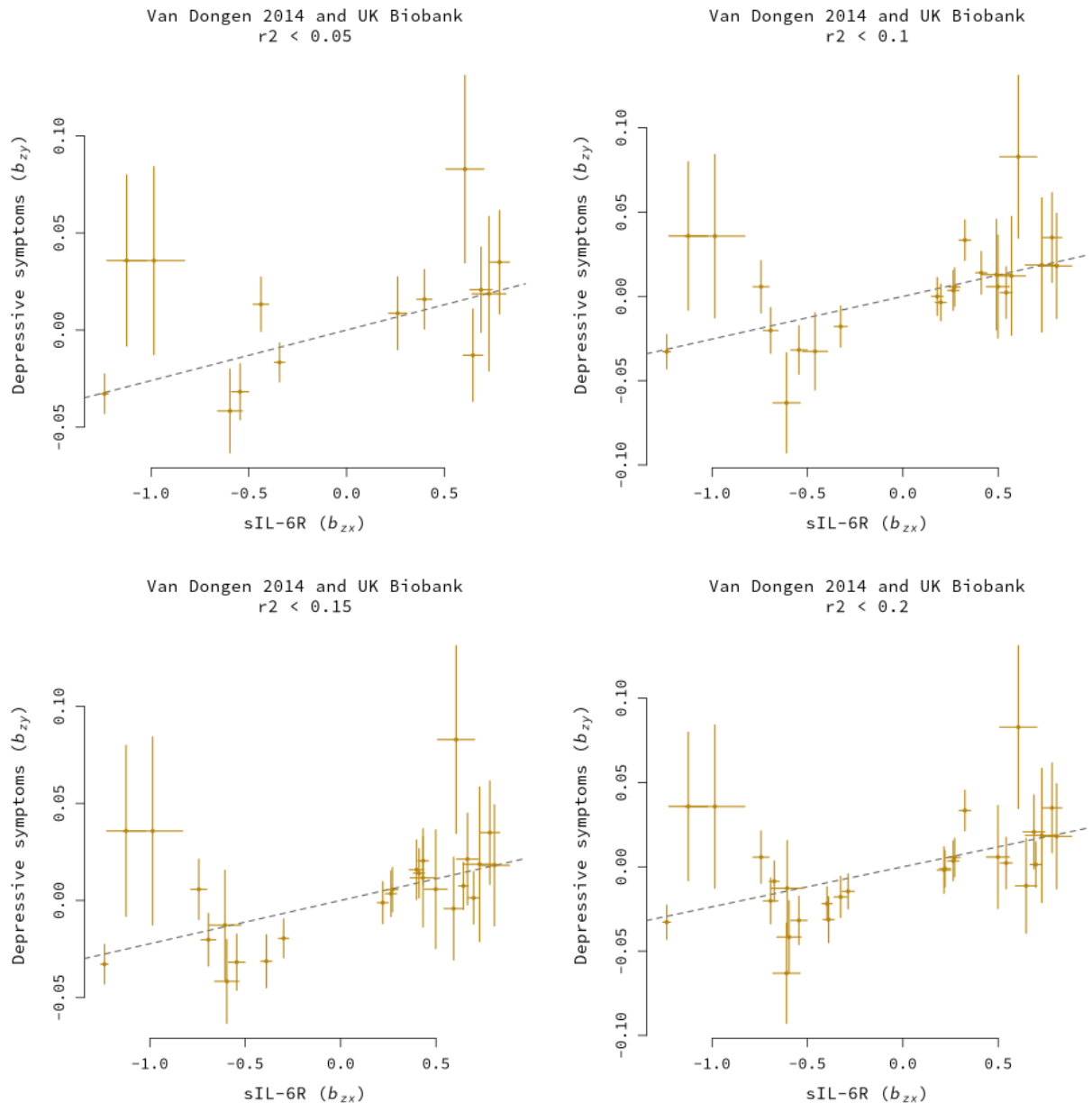
Figure C.8: Scatter plots for SNPs used in Maximum Likelihood analyses



For each combination of datasets, only the SNP selection method producing the largest number of SNPs is shown in the figures above. When GCTA-COJO was used for SNP selection, COJO-adjusted SNP betas and standard errors were used for the analysis.

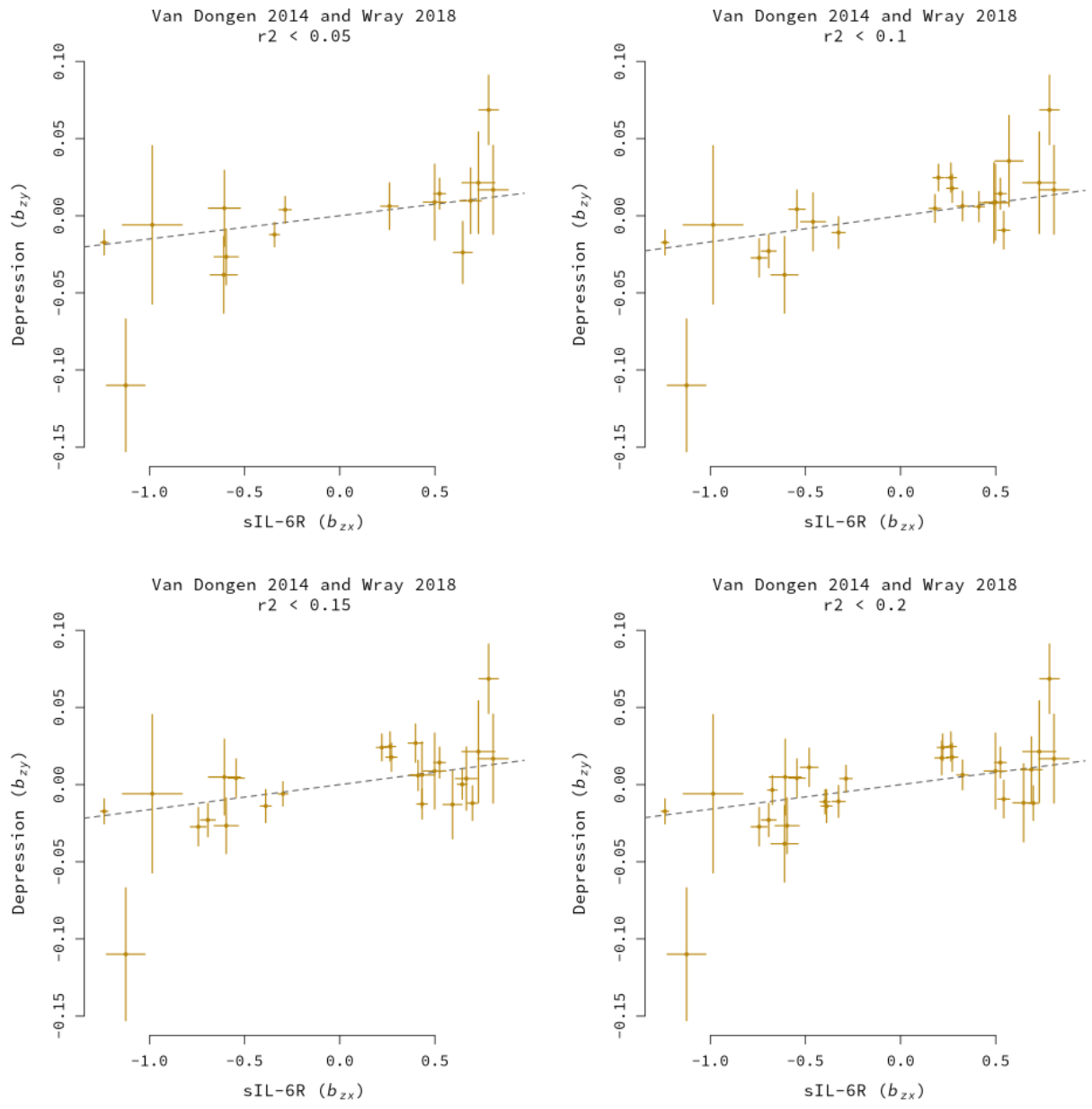
To examine the possibility of pleiotropy (in this context pleiotropy refers to an association between the SNP and the outcome via a mechanism other than the exposure), the MR Egger intercept was checked for significance, and was not found to be significant for any of the analyses performed in this study. A non-significant MR Egger intercept does not fully eliminate the possibility of pleiotropy, as discussed further in the paper text.

Figure C.9: Scatter plots for GSMR analyses using Van Dongen 2014 (sIL-6R) and UK Biobank (Recurrent Depressive Symptoms)



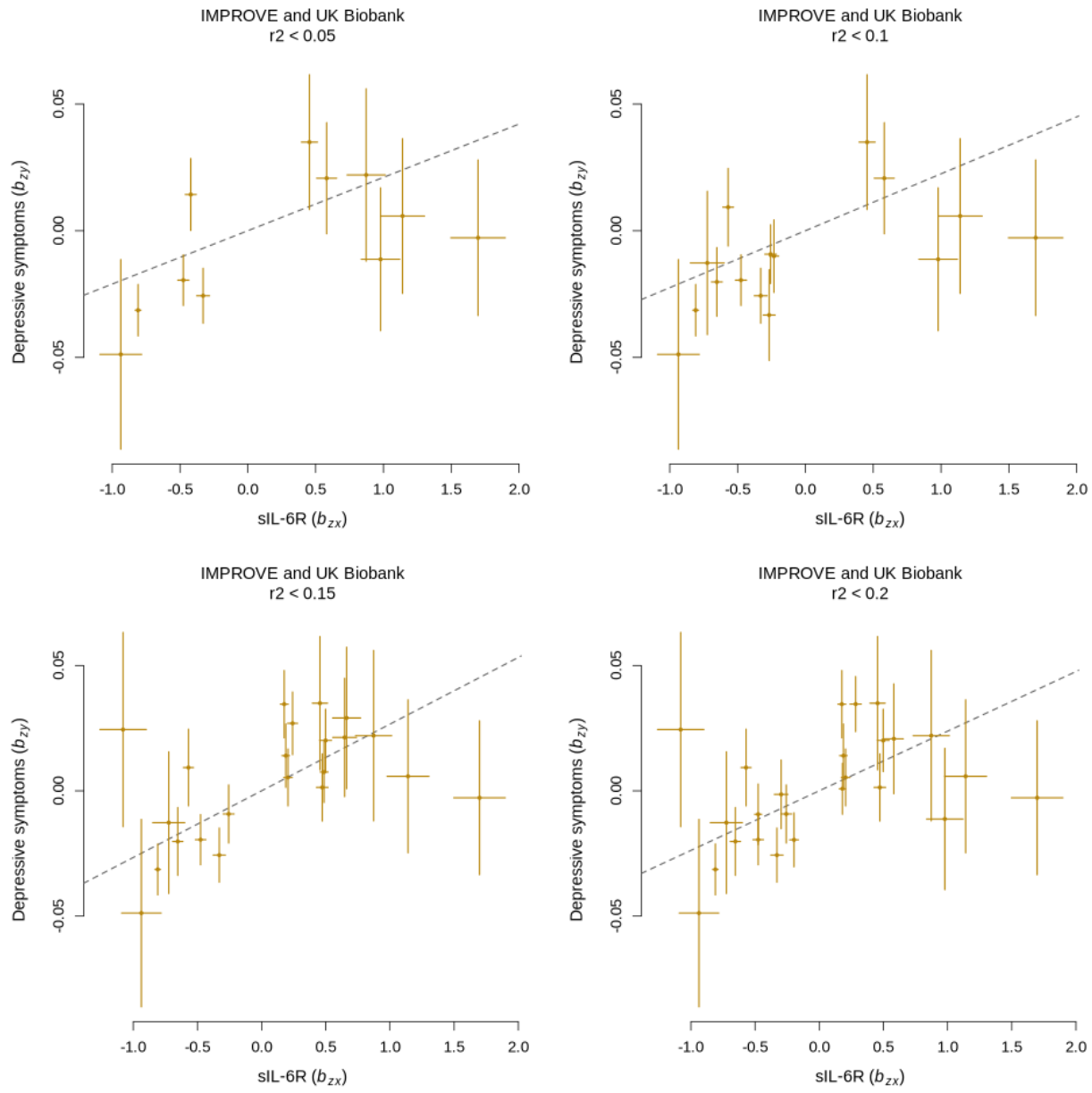
GSMR uses the HEIDI outlier test to examine the possibility of pleiotropy (in this context pleiotropy refers to an association between the SNP and the outcome via a mechanism other than the exposure). This test did not identify any potentially-pleiotropic SNPs for the analyses shown above. This does not fully eliminate the possibility of certain types of pleiotropy, as discussed further in the text.

Figure C.10: Scatter plots for GSMR analyses using Van Dongen 2014 (sIL-6R) and PGC MDD 2018 (Depression)



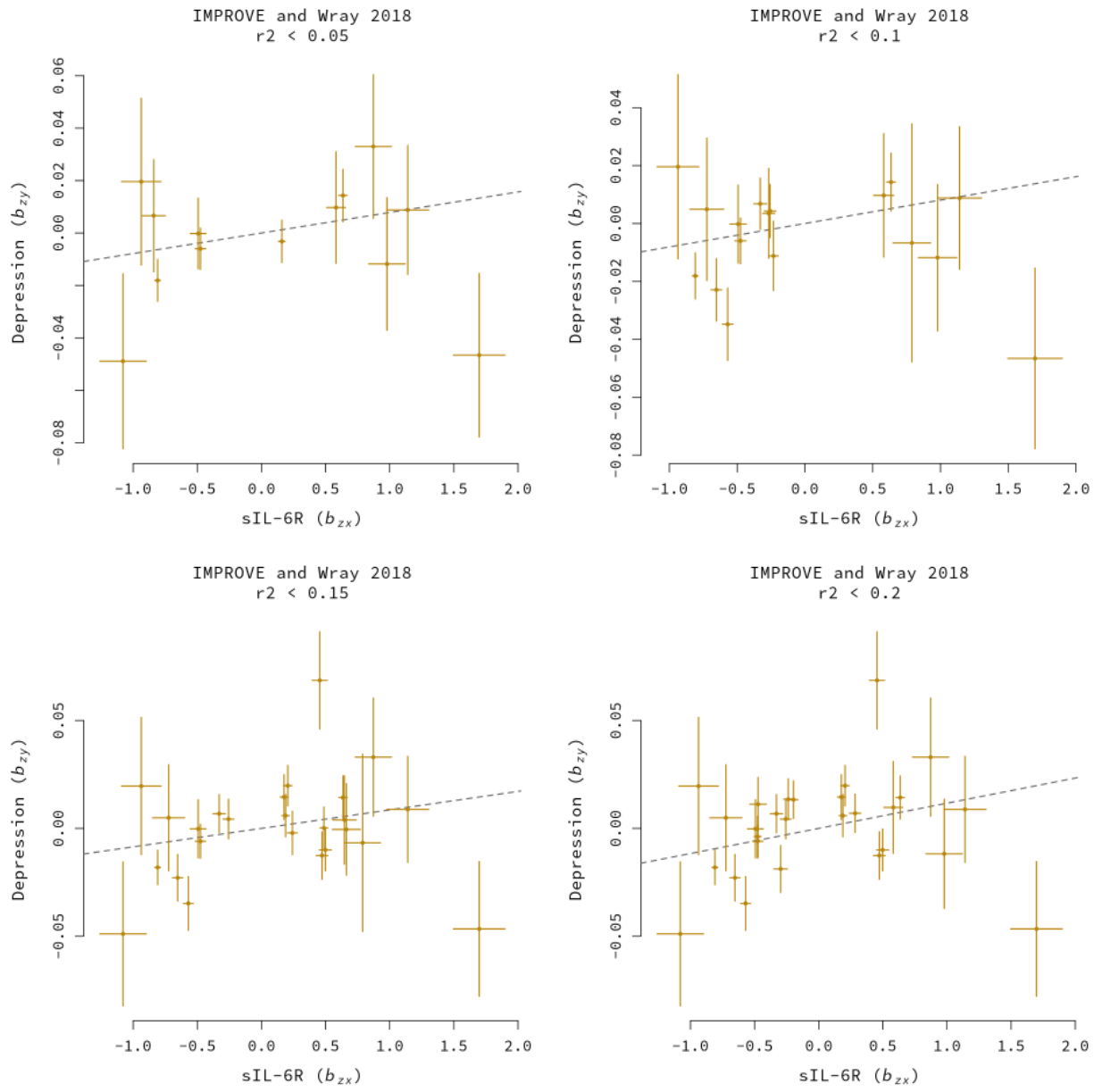
GSMR uses the HEIDI outlier test to examine the possibility of pleiotropy (in this context pleiotropy refers to an association between the SNP and the outcome via a mechanism other than the exposure). This test did not identify any potentially-pleiotropic SNPs for the analyses shown above. This does not fully eliminate the possibility of certain types of pleiotropy, as discussed further in the text.

Figure C.11: Scatter plots for GSMR analyses using IMPROVE (sIL-6R) and UK Biobank (Recurrent Depressive Symptoms)



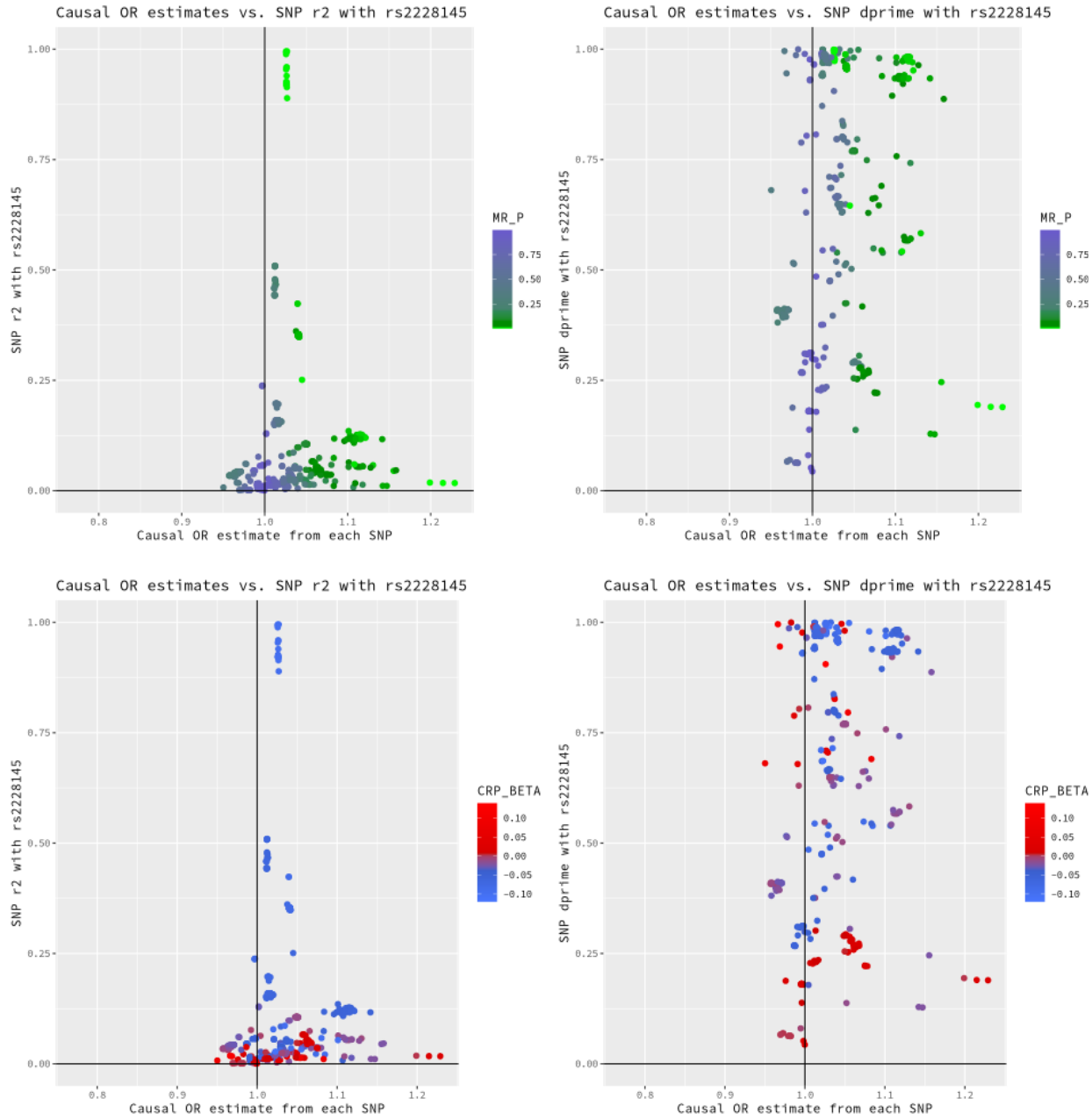
GSMR uses the HEIDI outlier test to examine the possibility of pleiotropy (in this context pleiotropy refers to an association between the SNP and the outcome via a mechanism other than the exposure). This test did not identify any potentially-pleiotropic SNPs for the analyses shown above. This does not fully eliminate the possibility of certain types of pleiotropy, as discussed further in the text.

Figure C.12: Scatter plots for GSMR analyses using IMPROVE (sIL-6R) and PGC MDD 2018 (Depression)



GSMR uses the HEIDI outlier test to examine the possibility of pleiotropy (in this context pleiotropy refers to an association between the SNP and the outcome via a mechanism other than the exposure). This test identified potentially-pleiotropic SNPs in the sets of SNPs produced when using r^2 clumping thresholds of 0.05 (rs12739228), 0.1 (rs12739228), and 0.2 (rs192423521). These SNPs were excluded from the GSMR analyses, and do not appear in the scatter plots above.

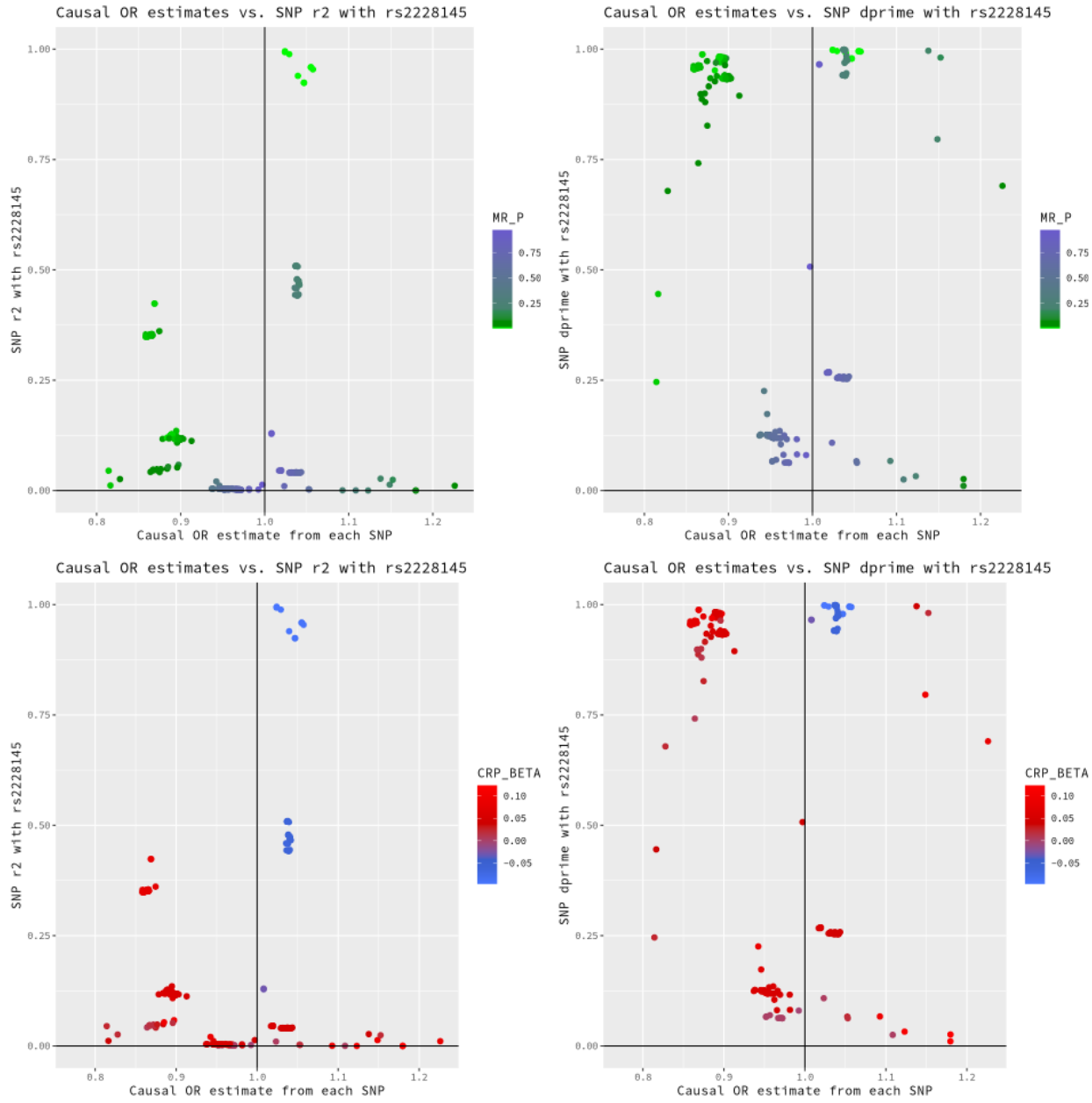
Figure C.13: Scatter plots using Van Dongen 2014 GWAS data to illustrate relationship between rs2228145 and other SNPs associated with sIL-6R



These scatter plots illustrate the relationships between rs2228145 and other SNPs associated with sIL-6R levels in the Van Dongen 2014 data. The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling.

Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using that SNP to estimate the effect of IL6R expression on depression (using the Wald ratio of coefficients method, and UK Biobank as the outcome sample). mr_P refers to the p-value produced by each SNP when used in the Wald ratio analysis. CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R.

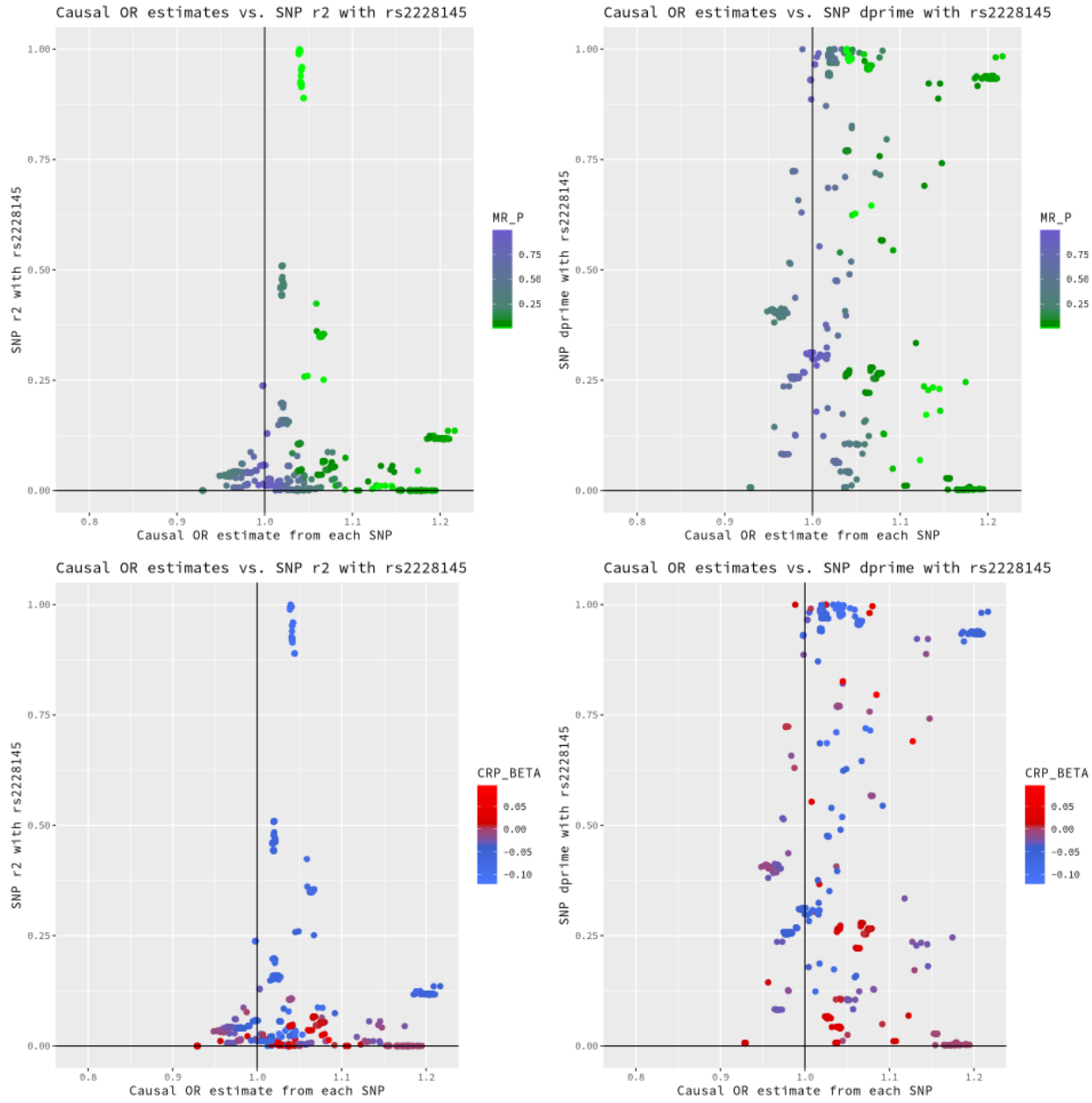
Figure C.14: Scatter plots using Van Dongen 2014 GWAS (conditional on rs2228145) to illustrate relationship between rs2228145 and other SNPs associated with sIL-6R



These plots illustrate that despite using GWAS results conditional on rs2228145, SNPs in closer LD with rs2228145 are still more likely to produce significant results and to be associated with lower classical IL-6 signaling. Although lower classical IL-6 signaling is a known effect of rs2228145, it is not expected for SNPs which increase sIL-6R levels via other mechanisms, suggesting that conditional analysis did not fully eliminate the effects of rs2228145.

The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling. Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using the Wald ratio of coefficients method and using UK Biobank as the outcome sample). CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R

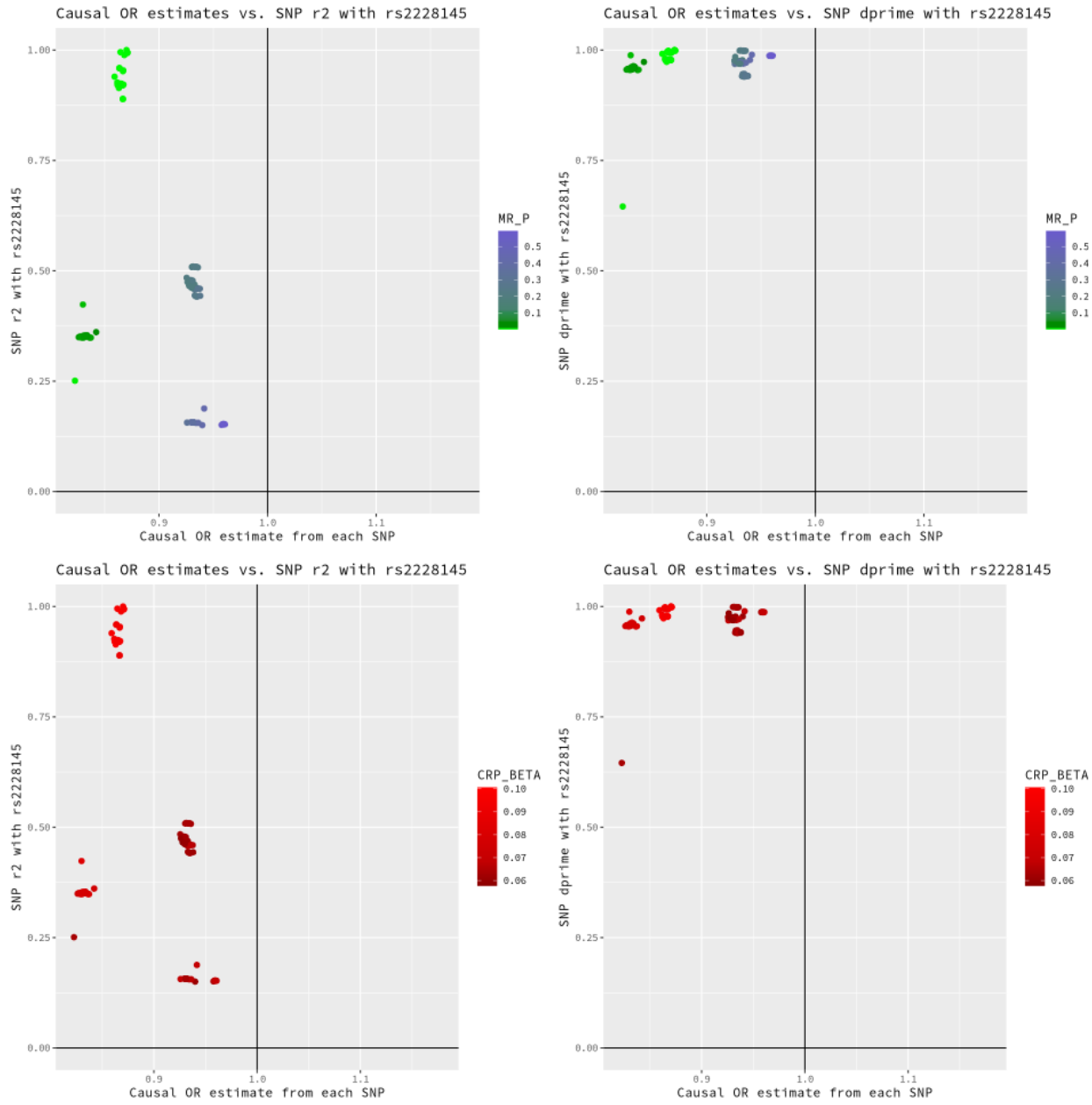
Figure C.15: Scatter plots using IMPROVE GWAS data to illustrate relationship between rs2228145 and other SNPs associated with sIL-6R



These scatter plots illustrate the relationships between rs2228145 and other SNPs associated with sIL-6R levels in the IMPROVE data. The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling.

Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using that SNP to estimate the effect of IL6R expression on depression (using the Wald ratio of coefficients method, and UK Biobank as the outcome sample). mr_P refers to the p-value produced by each SNP when used in the Wald ratio analysis. CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R.

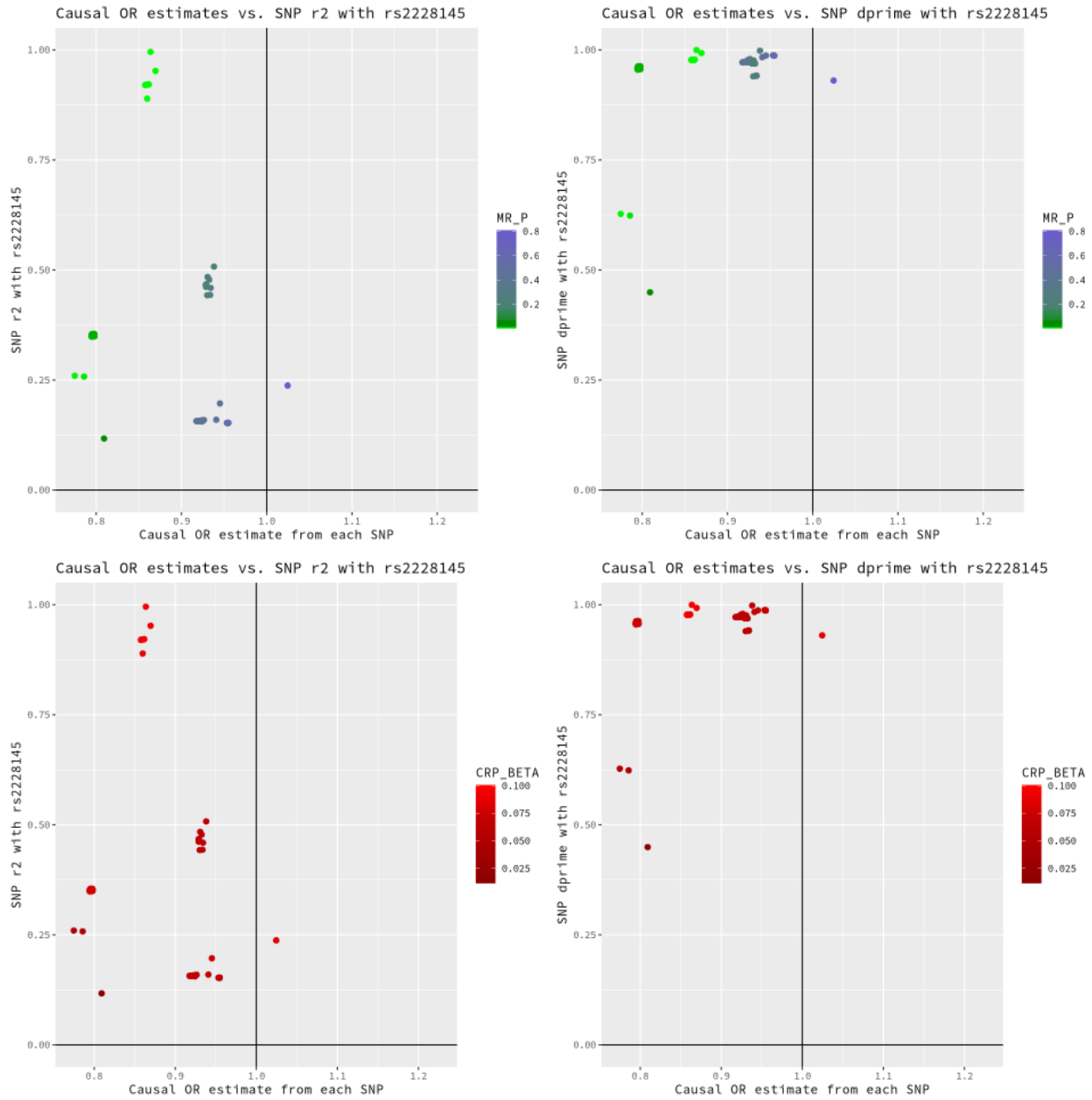
Figure C.16: Scatter plots examining the relationship between CAGE IL6R eQTLs and rs2228145



These scatter plots illustrate that eQTLs associated with higher IL6R expression in the CAGE data tend to have high |D'| values and moderate-to-high r2 values with rs2228145. Additionally, the alleles of these SNPs associated with increased IL6R expression also consistently have positive betas for their association with CRP (this is consistent with the effects of rs2228145, because the major allele of rs2228145 is associated with lower sIL-6R, higher CRP, and higher IL6R gene expression.)

The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling. Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using the Wald ratio of coefficients method and using UK Biobank as the outcome sample). CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R

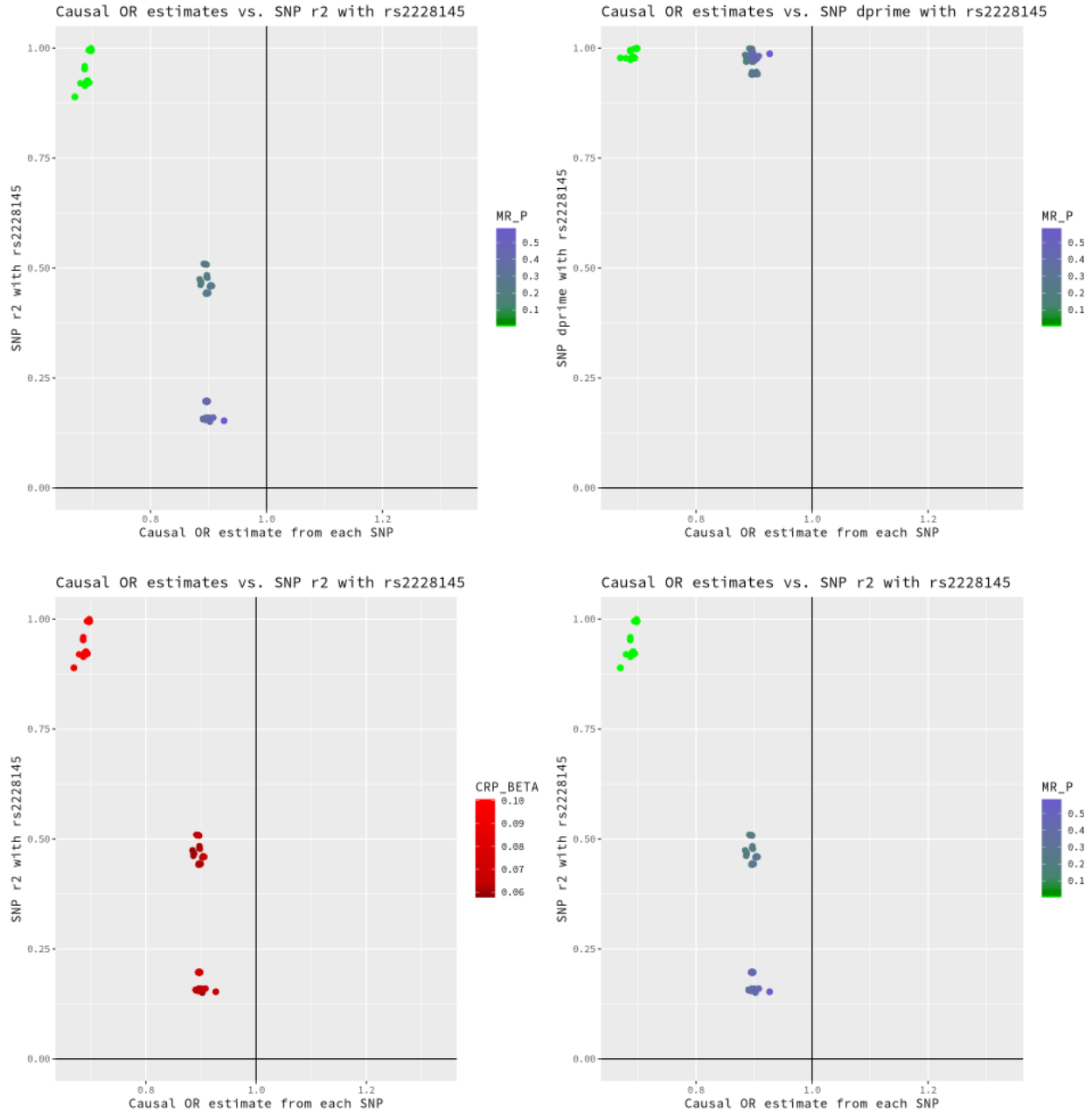
Figure C.17: Scatter plots examining the relationship between Westra 2013 IL6R eQTLs and rs2228145



These scatter plots illustrate that eQTLs associated with higher IL6R expression in the Westra 2013 data tend to have high |D'| values and moderate-to-high r2 values with rs2228145. Additionally, the alleles of these SNPs associated with increased IL6R expression also consistently have positive betas for their association with CRP (this is consistent with the effects of rs2228145, because the major allele of rs2228145 is associated with lower sIL-6R, higher CRP, and higher IL6R gene expression.)

The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling. Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using the Wald ratio of coefficients method and using UK Biobank as the outcome sample). CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R

Figure C.18: Scatter plots examining the relationship between GTEx IL6R blood eQTLs and rs2228145



These scatter plots illustrate that eQTLs associated with higher IL6R expression in the GTEx blood eQTL data tend to have high |D'| values and moderate-to-high r2 values with rs2228145. Additionally, the alleles of these SNPs associated with increased IL6R expression also consistently have positive betas for their association with CRP (this is consistent with the effects of rs2228145, because the major allele of rs2228145 is associated with lower sIL-6R, higher CRP, and higher IL6R gene expression.)

The top plots illustrate the strength of the estimate produced by each SNP (colored by P-value produced for the Wald ratio). In the bottom plots, CRP is used as a proxy for the association between each SNP and classical IL-6 signaling. Dprime refers to |D'|, Lewontin's absolute value d-prime. The causal OR estimate from each SNP refers to odds ratio produced when using the Wald ratio of coefficients method and using UK Biobank as the outcome sample). CRP beta values are from the KORA coefficients, with alleles harmonized so that the CRP beta shown is for the allele that increases sIL-6R.

Appendix D: Supplemental Tables and Figures for Chapter 4

Table D.1: Additional details of samples used in LD Score Regression

Phenotype	Source	Sample prevalence	Estimated population prevalence*	SNP Filtering[a]
Recurrent depressive symptoms	UK Biobank	36.1%	26.4% [b]	SNPs filtered according to QC steps for UK Biobank data described in supplemental note
Major depressive disorder	Wray 2018[194]	34.6%	14.6%[341]	SNPs filtered for MAF >= 1%, sample size at least 95% of total sample N, and no significant heterogeneity between cohorts (Cochran's Q p-value > $1*10^{-6}$)
Allergic rhinitis or eczema	UK Biobank	22.8%	22.9%[b]	SNPs filtered according to QC steps for UK Biobank data described in supplemental note
Asthma	UK Biobank	11.4%	11.5%[b]	SNPs filtered according to QC steps for UK Biobank data described in supplemental note
Allergic sensitization	Waage 2018[79]	33%	46.2%[342]	SNPs filtered for MAF >= 1%, sample size at least 95% of total sample N, and no significant heterogeneity between cohorts (Cochran's Q p-value > $1*10^{-6}$)
Atopic dermatitis	Paternoster 2015[262]	26.4%	10%[262]	SNPs filtered for MAF >= 1%, sample size at least 80% of total sample N, and no significant heterogeneity between cohorts (Cochran's Q p-value > $1*10^{-6}$)

* Estimated population prevalence, used to convert heritability estimates from observed scale to liability scale. This prevalence is used only for heritability/co-heritability, not for genetic correlation.

[a] All SNPs were also filtered by the LD Score Regression software to exclude duplicates, non-SNPs, SNPs with more than two alleles, stand ambiguous SNPs, and SNPs not present in the 1000 genomes EUR LD reference sample

[b] Based on prevalence in all white individuals with British ancestry in the UK Biobank sample

Table D.2: Estimated heritability and co-heritability from LD Score Regression

	Recurrent Depressive symptoms	**Allergies/eczema**	**Asthma**	**Major Depressive Disorder**	**Allergic sensitization**	**Atopic dermatitis**
Data source	UK Biobank	UK Biobank	UK Biobank	PGC	EAGLE	EAGLE
Recurrent Depressive symptoms	0.115 (0.007)	0.139 (0.011)	0.153 (0.016)	0.103 (0.006)	0.254 (0.046)	0.116 (0.009)
Allergies/eczema	0.139 (0.011)	0.130 (0.009)	0.153 (0.016)	0.139 (0.010)	0.254 (0.047)	0.143 (0.014)
Asthma	0.153 (0.016)	0.153 (0.016)	0.142 (0.013)	0.155 (0.016)	0.254 (0.046)	0.158 (0.021)
Major Depressive Disorder (PGC)	0.103 (0.006)	0.139 (0.010)	0.155 (0.016)	0.102 (0.006)	0.253 (0.043)	0.103 (0.007)
Allergic sensitization (EAGLE)	0.254 (0.046)	0.254 (0.047)	0.254 (0.046)	0.253 (0.043)	0.248 (0.044)	0.248 (0.054)
Atopic dermatitis (EAGLE)	0.116 (0.009)	0.143 (0.014)	0.158 (0.021)	0.103 (0.007)	0.248 (0.054)	0.104 (0.024)

Table D.2 shows estimated trait heritabilities (diagonals) and co-heritabilities (off-diagonal) when excluding the MHC region. Results are given as percent (SD).

Table D.3: Cross-trait genetic correlations when including the Major Histocompatibility Complex

	Depressive symptoms	**Allergic rhinitis or eczema**	**Asthma**	**Major Depressive Disorder**	**Allergic sensitization**	**Atopic dermatitis**
Data source	UK Biobank	UK Biobank	UK Biobank	PGC MDD 2018	EAGLE	EAGLE
Depressive symptoms	--	0.176 (p=1.7e-08)	0.146 (p=3.0e-04)	0.860 (p=2.9e-122)	-0.131 (p=0.061)	-0.052 (p=0.573)
Allergic rhinitis or eczema	0.176 (p=1.7e-08)	--	0.667 (p=4.5e-87)	0.010 (p=0.746)	0.757 (p=1.2e-37)	0.513 (p=4.0e-13)
Asthma	0.146 (p=3.0e-04)	0.667 (p=4.5e-87)	--	0.208 (p=9.4e-07)	0.579 (p=2.0e-17)	0.400 (p=8.5e-07)
Major Depressive Disorder	0.860 (p=2.9e-122)	0.010 (p=0.746)	0.208 (p=9.4e-07)	--	-0.053 (p=0.414)	0.072 (p=0.340)
Allergic sensitization	-0.131 (p=0.061)	0.757 (p=1.2e-37)	0.579 (p=2.0e-17)	-0.053 (p=0.414)	--	0.421 (p=8.0e-04)
Atopic dermatitis	-0.052 (p=0.573)	0.513 (p=4.0e-13)	0.400 (p=8.5e-07)	0.072 (p=0.340)	0.421 (p=8.0e-04)	--

Table D.3 shows results for genetic correlations between depressive and atopic phenotypes, with the Major Histocompatibility Complex excluded.

Table D.4: Trait co-heritabilities when including the Major Histocompatibility Complex

	Recurrent Depressive symptoms	**Allergies/eczema**	**Asthma**	**Major Depressive Disorder**	**Allergic sensitization**	**Atopic dermatitis**
Data source	UK Biobank	UK Biobank	UK Biobank	PGC	EAGLE	EAGLE
Recurrent Depressive symptoms	0.117 (0.008)	0.141 (0.011)	0.162 (0.018)	0.105 (0.006)	0.261 (0.045)	0.118 (0.010)
Allergies/eczema	0.141 (0.011)	0.132 (0.009)	0.162 (0.018)	0.141 (0.011)	0.261 (0.045)	0.146 (0.014)
Asthma	0.162 (0.018)	0.162 (0.018)	0.150 (0.015)	0.163 (0.019)	0.261 (0.045)	0.165 (0.023)
Major Depressive Disorder (PGC)	0.105 (0.006)	0.141 (0.011)	0.163 (0.019)	0.105 (0.006)	0.261 (0.044)	0.105 (0.007)
Allergic sensitization (EAGLE)	0.261 (0.045)	0.261 (0.045)	0.261 (0.045)	0.261 (0.044)	0.256 (0.042)	0.253 (0.055)
Atopic dermatitis (EAGLE)	0.118 (0.010)	0.146 (0.014)	0.165 (0.023)	0.105 (0.007)	0.253 (0.055)	0.104 (0.024)

Table D.4 shows estimated trait heritabilities (diagonals) and co-heritabilities (off-diagonal) when including the MHC region. Results are given as percent (SD).

Table D.5: Polygenic risk score characteristics

Score	Base sample	Target sample	P-value threshold for SNP inclusion	# SNPs
Allergic Sensitization	Waage 2018 (6988 cases / 16,420 controls)	UK Biobank[a] (34342 cases / 129303 controls)	5.005e-05	112
UKB large	UK Biobank[a] (30342 cases / 125303 controls)	UK Biobank[a] (4000 cases / 4000 controls)	0.00015005	718
UKB strict	UK Biobank[b] (11636 cases / 63561 controls)	UK Biobank[b] (4000 cases / 4000 controls)	5.005e-05	199

[a] UK Biobank individuals from the non-overlapping atopic disorders sample, using the allergy/eczema phenotype

[b] UK Biobank individuals from the non-overlapping atopic disorders sample, using the allergy/eczema phenotype, who screened negative for any depressive symptoms in two weeks preceding interview

Table D.6: Polygenic risk score performance

Score	Score performance (target sample)			Score performance (analytic sample)			Association with depression (analytic sample)		
	R^2	OR (95% CI)	p	R^2	OR (95% CI)	p	R^2	OR (95% CI)	p
Allergic sensitization	0.0056	1.16 (1.14-1.17)	6.78e-129	0.0060	1.16 (1.14-1.17)	3.40e-111	0.0000	1.00 (0.99-1.01)	0.7336
UKB large	0.0181	1.27 (1.21-1.33)	4.05e-25	0.0174	1.29 (1.28-1.31)	7.43e-319	0.0001	1.01 (1.00-1.03)	0.0257
UKB strict	0.0149	1.24 (1.19-1.30)	5.53e-21	0.0071	1.18 (1.16-1.19)	5.91e-133	0.0000	1.01 (1.00-1.02)	0.2061

Table D.6 Shows the performance of each polygenic risk score in the target and analytic samples. All three scores showed highly significant associations with the Allergy/Eczema phenotype in the target and analytic samples, but produced fairly low R^2 values (R^2 = 0.0056 - 0.0181).

Table D.7: Summary of each analysis

	LD Score Regression	Mendelian Randomization	Polygenic Risk Score comparison
Objective(s)	To examine shared genetic liability between depression and atopic disorders	To assess whether atopic disorders have a causal effect of depression	1. To assess whether the association between atopy and depression may be a spurious association arising from differential self-reporting or somatization by depression cases. 2. To assess whether depression may increase liability to atopy, allowing individuals with lower genetic liability to atopy to develop atopic phenotypes.
Hypothesis	There are shared genetic risk factors that contribute to both atopy and depression	Atopic disorders have a causal effect on depression	Among individuals with self-reported allergies/eczema, those with a history of depression will have lower atopy PRS than those without a history of depression.
Depression phenotype(s)	• "Recurrent Depressive Symptoms" (UK Biobank "complete depression data" sample) • "Major Depressive Disorder" (PGC MDD 2018)	• "Recurrent Depressive Symptoms" (UK Biobank "complete depression data" sample) • "Major Depressive Disorder" (PGC MDD 2018)	• "Recurrent Depressive Symptoms" (UK Biobank "complete depression data" sample)
Atopic phenotype(s)	• "Allergies/Eczema" (UK Biobank "full" sample) • "Asthma" (UK Biobank "full" sample) • "Allergic Sensitization" (EAGLE) • "Atopic dermatitis" (EAGLE)	• "Allergies/Eczema" (UK Biobank "atopic phenotypes only" sample) • "Asthma" (UK Biobank "atopic phenotypes only" sample) • "Allergic Sensitization" (EAGLE) • "Atopic dermatitis" (EAGLE)	• "Allergies/Eczema" (UK Biobank "atopic phenotypes only" sample) • "Allergic Sensitization" (EAGLE)
Result	Findings support shared genetic liability between depression phenotypes and asthma, and potentially also allergies/eczema.	No evidence to support a causal relationship of any atopic phenotype on depression.	Weak/suggestive evidence that among allergy/eczema cases, those with depression may have lower atopy PRSs. Replication necessary, and if replicated would need further study to distinguish between potential explanations for the difference.

Supplemental Figures

Figure D.1: Forest plots for Mendelian Randomization SNPs using the "Recurrent Depressive Symptoms" phenotype (UK Biobank)

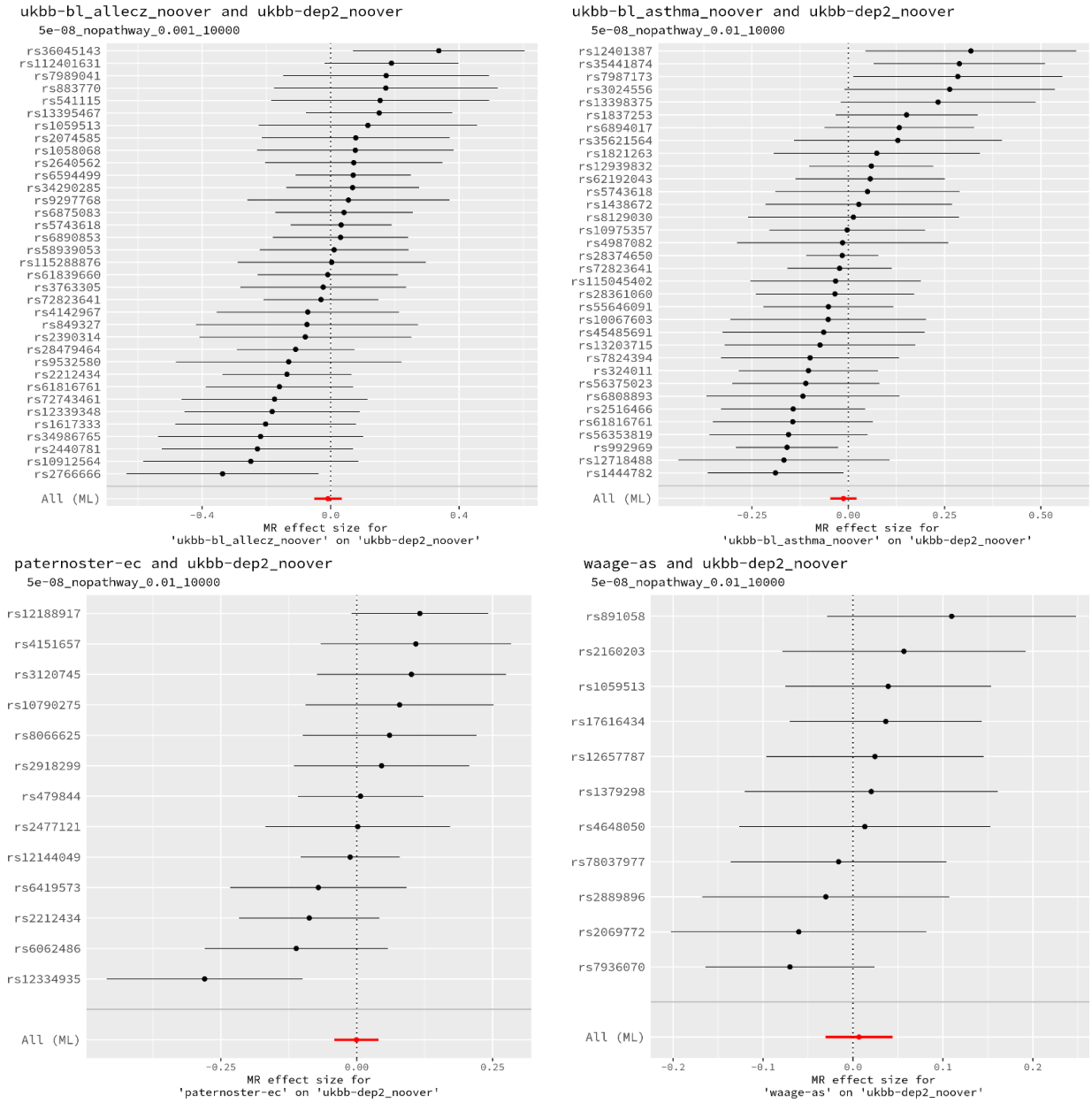


Figure D.2: Leave-one-SNP-out plots for Mendelian Randomization SNPs using the "Recurrent Depressive Symptoms" phenotype (UK Biobank)

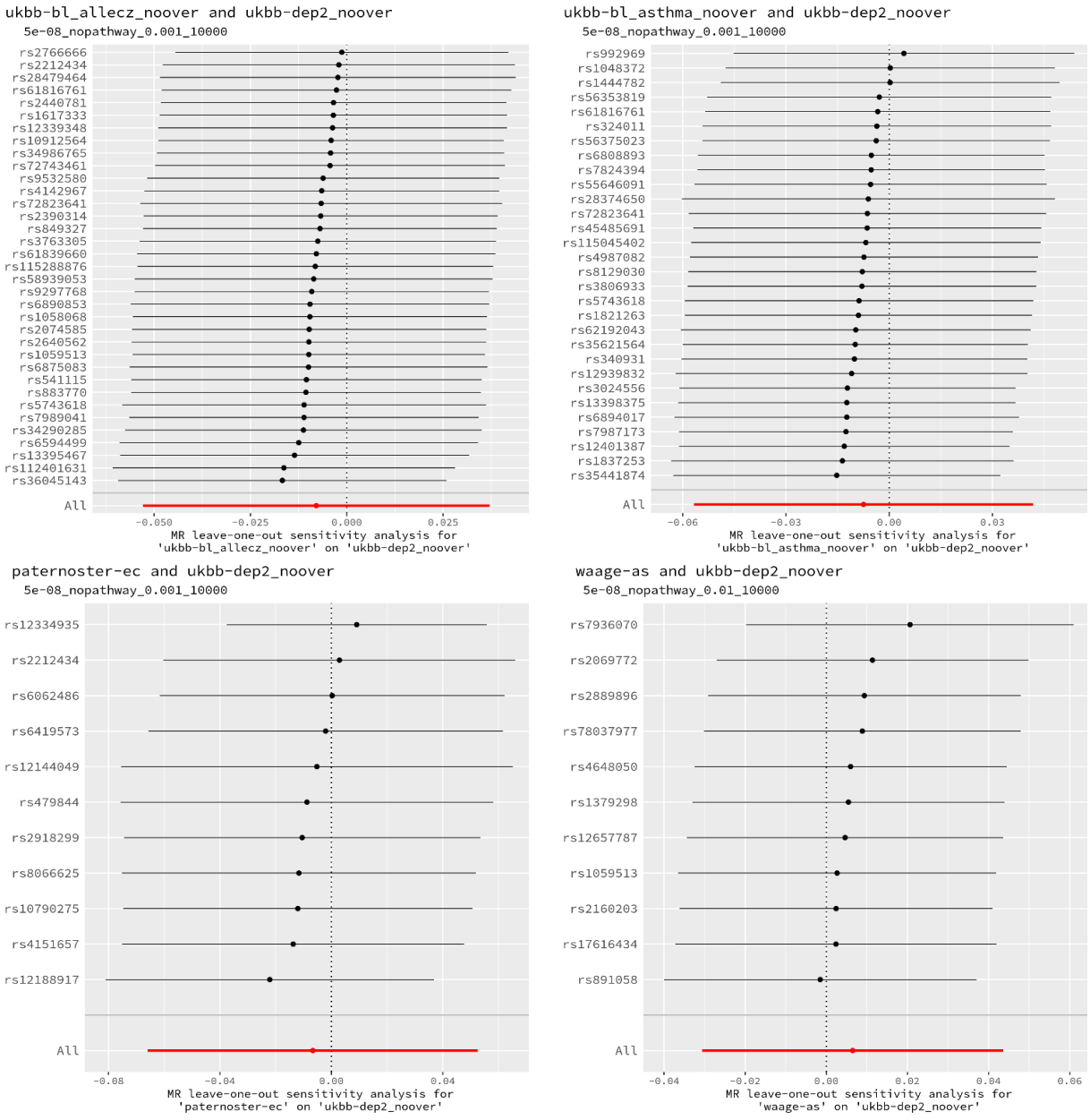
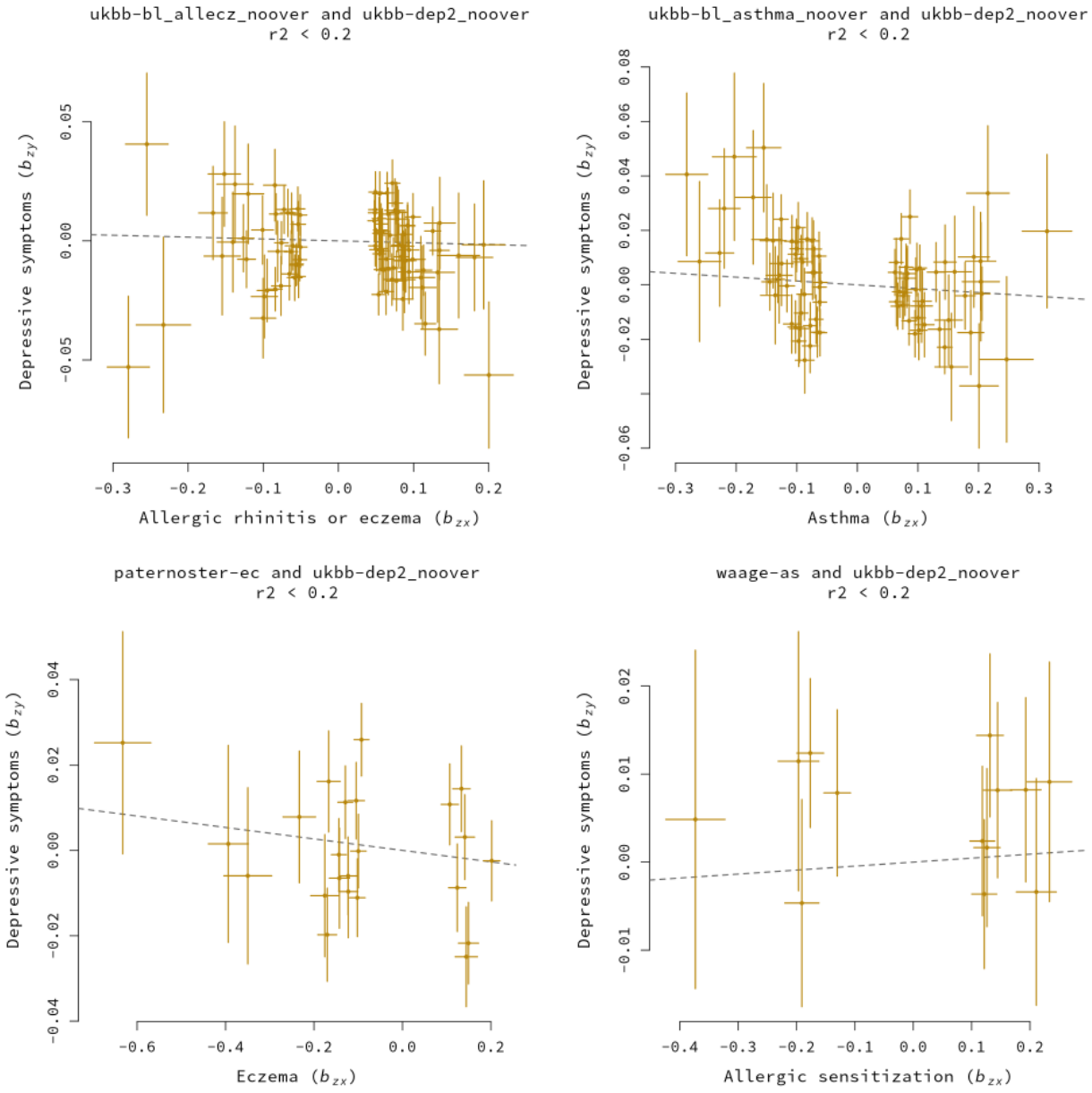


Figure D.3: GSMR plots for the "Recurrent Depressive Symptoms" phenotype (UK Biobank)



The HEIDI outlier test excluded the following SNPs for the asthma analysis: rs2647074, rs9268805, rs4990036, rs2302776. These SNPs are not shown in the scatter plot above.

Figure D.4: Forest plots for Mendelian Randomization SNPs using the "Major Depressive Disorder" phenotype (PGC MDD 2018)

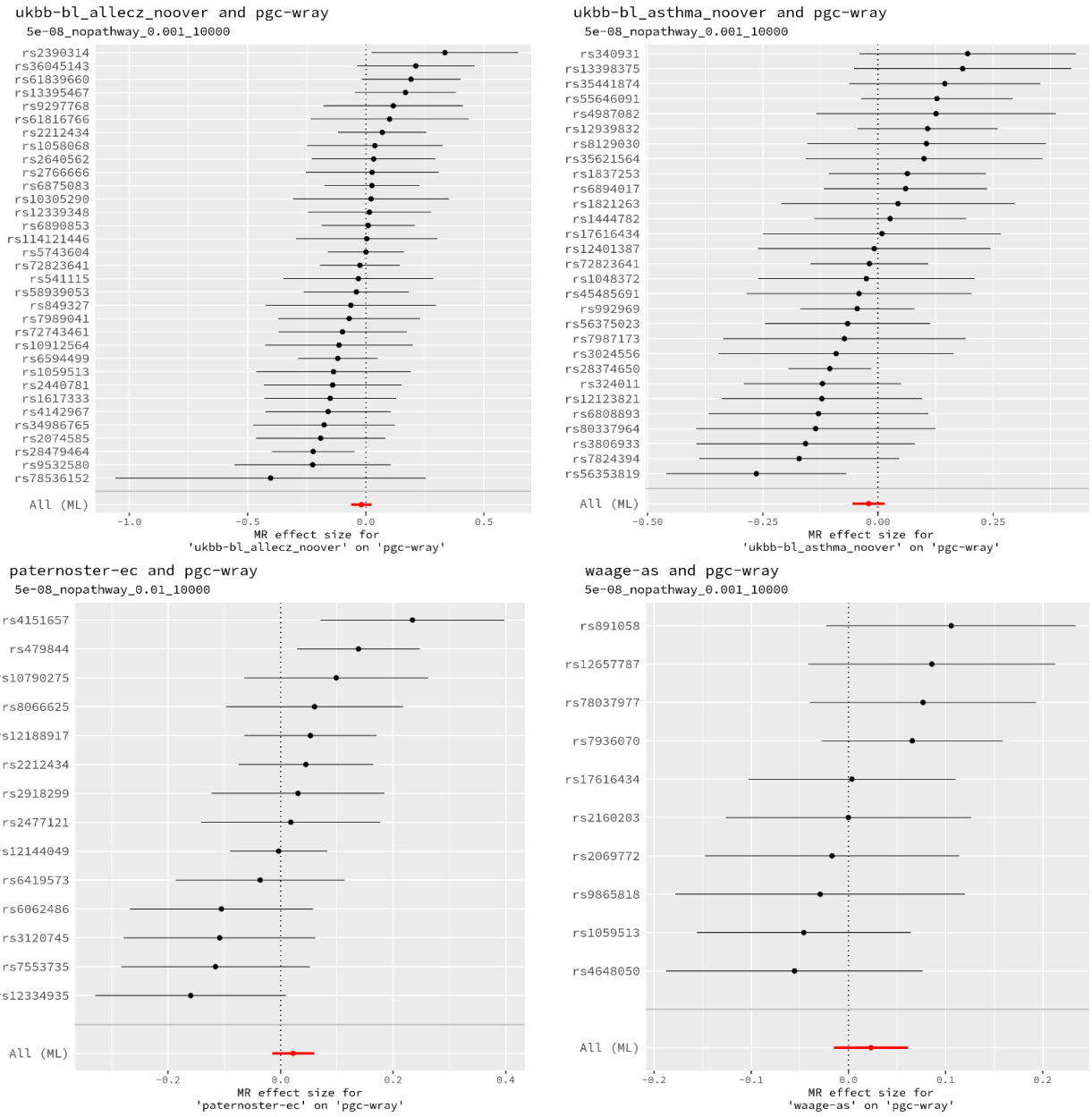


Figure D.5: Leave-one-SNP-out plots for Mendelian Randomization SNPs using the "Major Depressive Disorder" phenotype (PGC MDD 2018)

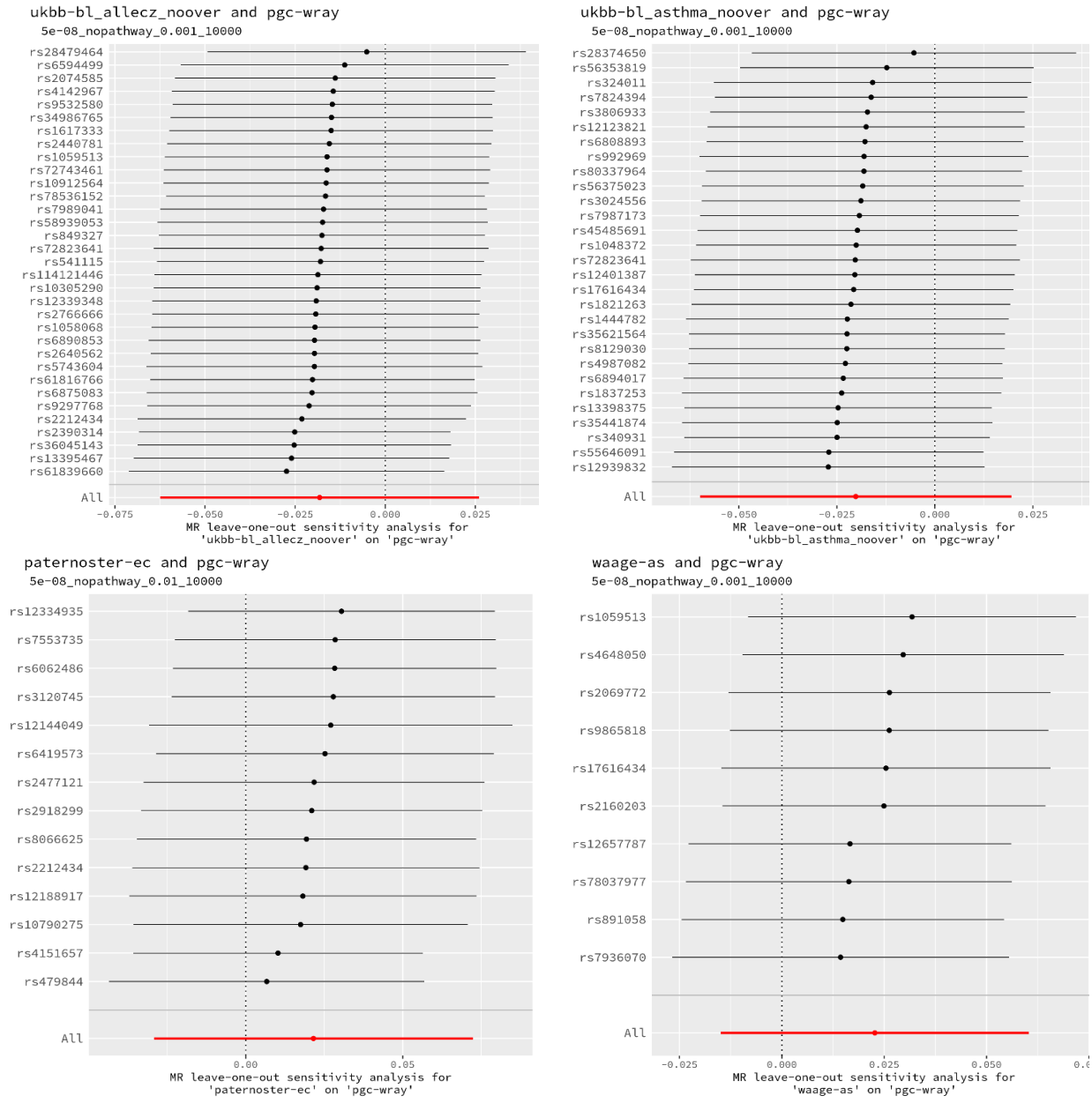
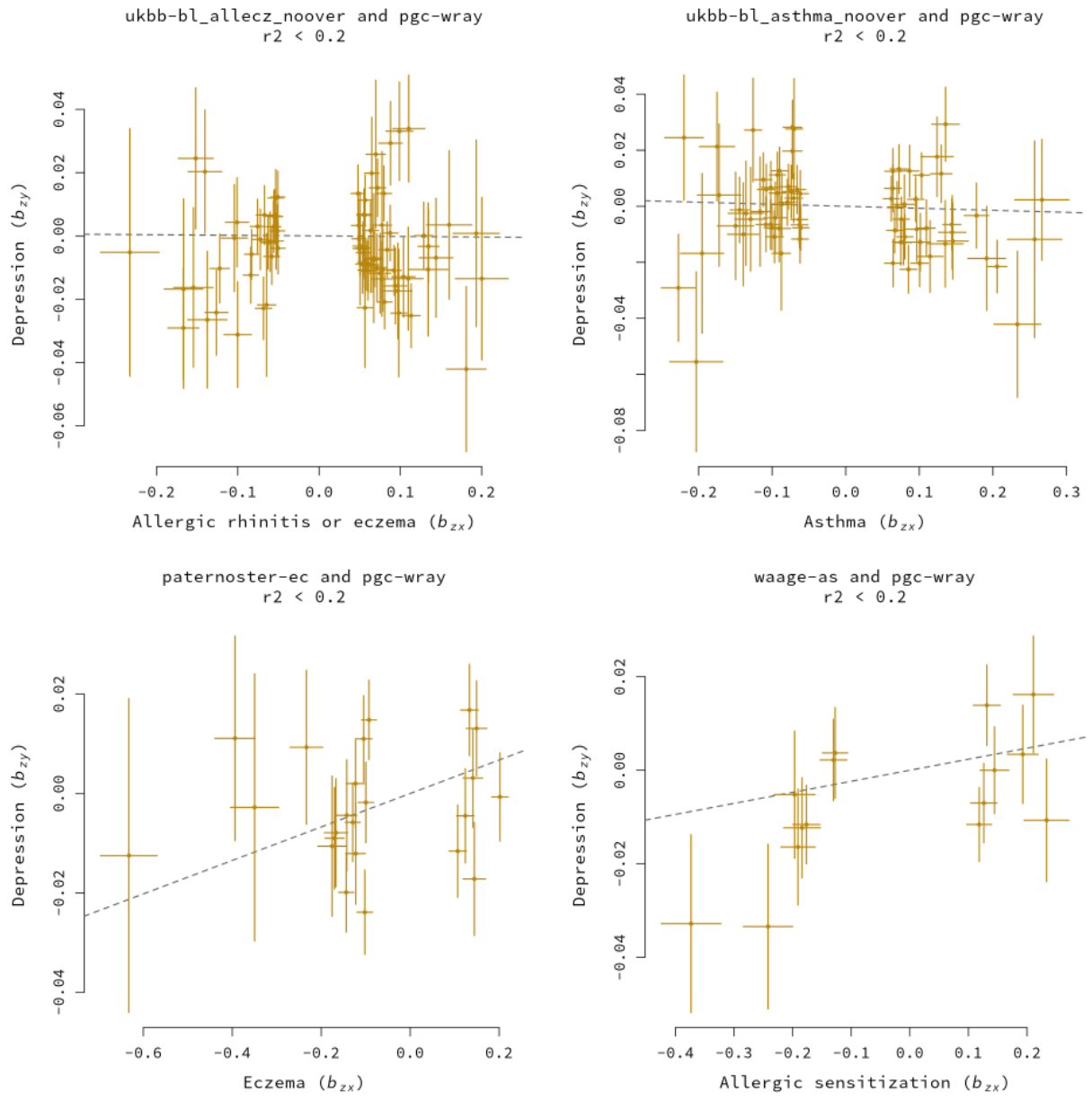
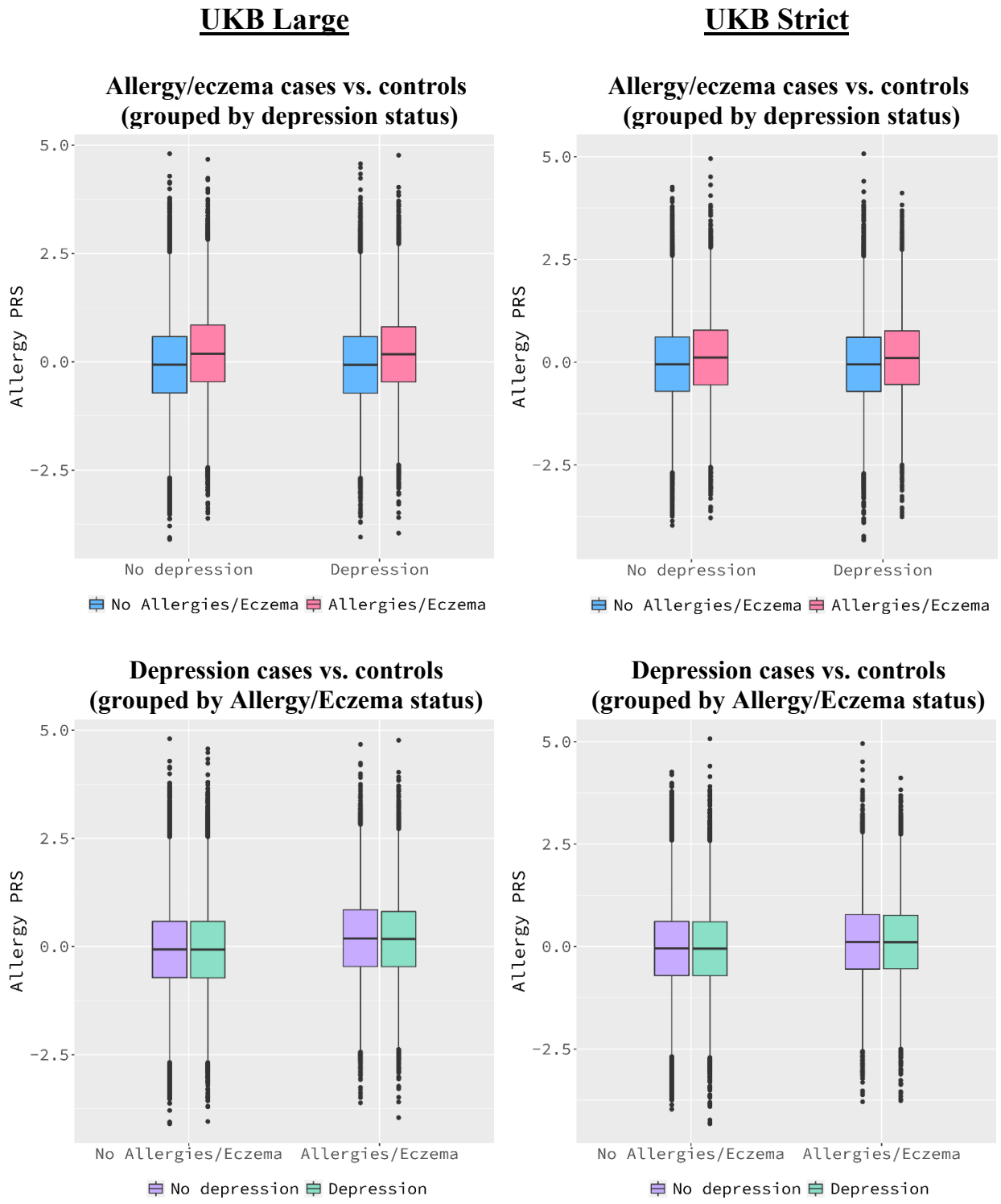


Figure D.6: GSMR scatter plots for the "Major Depressive Disorder" phenotype (PGC MDD 2018)



The HEIDI outlier test excluded the following SNPs for the asthma analysis: rs7041526, rs2855812. These SNPs are not shown in the scatter plot above.

Figure D.7: Polygenic risk score comparisons for the "UKB large" and "UKB strict" scores for the Allergy/Eczema phenotype



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