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List of abbreviations

BBB blood brain barrier

BMI body mass index

CCL C-C chemokine ligand

CNS central nervous system

DAMP damage-associated molecular pattern

DSM Diagnostic and Statistical Manual of Mental Disorders

FGL fused graphical least absolute shrinkage and selection operator

Fig Figure

GAD generalised anxiety disorder

gp130 glycoprotein 130

GR glucocorticoid receptor

GWAS genome-wide association study

HPA hypothalamus-pituitary-adrenal

IFN interferon

IL interleukin

IV instrumental variable

IVW inverse variance weighted

LD linkage disequilibrium

LPS lipopolysaccharide

NEGR1 neuronal growth regulator 1

NLPR NOD-like receptor

mAb monoclonal antibody

MARS Munich Antidepressant Response Signature study

MCP monocyte chemoattractant protein

MDD major depressive disorder

MR Mendelian randomisation

NLPR NOD-like receptor

NSAID non-steroidal anti-inflammatory drug

PAMP pathogen-associated molecular pattern

PRR pattern recognition receptor

PRS polygenic risk score

RCT randomised controlled trial

SMD standardised mean difference

SNP single nucleotide polymorphism

STAR*D Sequenced Treatment Alternatives to Relieve Depression study

T_H T helper cell

T_{reg} regulatory T cell

TNF tumour necrosis factor

WEIRD Western Educated Industrialised Rich Democratic

List of publications

Peer-reviewed articles

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1. Introductory Summary

Major depressive disorder (MDD) is a debilitating mental disorder: It is one of the leading causes of years lived with disability, second only to low back pain (Global Burden of Disease Study 2013 Collaborators, 2015). Worldwide, it affects around 6% of individuals each year, 20% of individuals during their lifetime, and women about twice as often as men. Current treatments for MDD primarily include monoaminergic drugs and psychotherapy, but about a third of patients do not respond to these treatments (Rush et al., 2006). With a population of 83 million people (Statistisches Bundesamt, 2021), this means in Germany alone over 1.5 million people will suffer from a chronic form of depression annually with no effective treatments available. These numbers emphasise that identifying new treatments for this large number of patients is a crucial public health objective that could reduce tremendous amounts of personal suffering and associated health care costs. Achieving this public health objective, however, requires a better characterisation and pathophysiological understanding of depression.

Diagnostically, depression can be diagnosed in one of two ways. On one side, patients can be diagnosed with MDD according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5). This requires the presence of at least one of two core symptoms of depressed mood or anhedonia and four or more of seven other symptoms including fatigue, feelings of guilt, changes in appetite, psychomotor changes, sleeping problems, cognitive problems, and suicidality (American Psychiatric Association, 2013). Importantly, these symptoms have to be present over a period of two weeks to qualify for the MDD diagnosis and they cannot be the result of another medical condition. The absence of a comorbid medical condition sets a clear dividing line for the other side of the diagnostic dichotomy, whereby depression is diagnosed under the umbrella of 'comorbid depression' in the context of a concurrent 'physical' illness such as rheumatoid arthritis, hepatitis, or diabetes (Gold et al., 2020; Smolen et al., 2018). Historically, comorbid depression has been considered to be a psychosocial consequence to the debilitating physical illness symptoms or as a consequence of treatment. However, several lines of evidence suggest that common factors contribute both to MDD and comorbid depression. Identifying and characterising these factors is a key research priority since it could open up the opportunity for new treatments targeting patients in both diagnostic groups. The focus of this thesis is one such factor, namely systemic inflammation, which has been implicated in depression by an accumulating amount of research over the past decades and may act akin to a "friendly fire", whereby the body's own defences exhibit detrimental off-target effects.

1.1 Mechanistic links connecting the immune system and the brain

If systemic inflammation was an important risk factor in depression, this would require plausible mechanistic links connecting the immune system and the brain. Contrary to the historical notion of these systems as independent and shielded by the blood-brain-barrier (BBB), it is well known today that there is cross-talk between the immune system and the brain via multiple pathways and key examples of immune-brain cross-talk are summarised next (Dantzer, O'Connor, Freund, Johnson, & Kelley, 2008; Galea, Bechmann, & Perry, 2007). Pro- and anti-inflammatory cytokines, which constitute the main messengers of the immune system, can relay signals through afferent nerves such as the vagus nerve. Cytokines can also enter the brain through volume diffusion in circumventricular zones outside of the BBB, via active BBB transport, or via local production from periventricular macrophages (Dantzer et al., 2008). In the brain, immune activity is also regulated through microglia that make up about 5-10% of central nervous system (CNS) cells. Microglia assume the role of CNS-resident macrophages and can be activated to combat tissue damage and to recognise and respond to emerging pathogens (Mondelli, Vernon, Turkheimer, Dazzan, & Pariante, 2017). In addition to cytokine signals from the periphery, these microglia can also produce cytokines locally and activity of pro-inflammatory cytokines has established effects on learning and plasticity processes, cognitive function, and behaviours. This has highlighted the importance of considering immune and brain functioning together as there are several plausible ways how inflammation can influence brain functioning and ultimately depression (Dantzer, 2001; McAfoose & Baune, 2009).

1.2 Inflammation and depression

1.2.1 Evidence from cross-sectional studies

Beyond mechanistic links, multiple lines of evidence have also specifically implicated systemic inflammation in depression. First, depression prevalence is much higher in chronic inflammatory illnesses as compared to the general population. Compared to the point prevalence of MDD in the general population of approximately 6%, the prevalence of comorbid MDD in chronic inflammatory illnesses is about 39% for rheumatoid arthritis, 22% for inflammatory bowel disease, and 28% for psoriasis (Gold et al., 2020; Otte et al., 2016).

Second, a wealth of cross-sectional case-control studies has indicated that circulating acute-phase reactants such as C-reactive protein (CRP), pro-inflammatory cytokines and chemokines are upregulated in patients with MDD as compared to healthy individuals

and these studies have been summarised in multiple systematic reviews and meta-analyses (Dowlati et al., 2010; Goldsmith, Rapaport, & Miller, 2016; Haapakoski, Mathieu, Ebmeier, Alenius, & Kivimäki, 2015; Howren, Lamkin, & Suls, 2009; Köhler et al., 2017). Most prominently, these studies have indicated an upregulation of CRP, which has been the most frequently studied inflammatory marker, and meta-analytic findings suggest that 21-34% of patients with depression have elevated levels of CRP according to the common medical cut-off of >3mg/L (Osimo, Baxter, Lewis, Jones, & Khandaker, 2019). Among cytokines, Köhler *et al.* (2017) have conducted the most comprehensive systematic review and meta-analysis, including 82 case-control studies reporting data on 3,212 MDD patients and 2,798 healthy controls. Their findings most reliably implicated the pro-inflammatory cytokines interleukin (IL)-6 and tumour necrosis factor (TNF)-α in depression, but additionally showed elevations of IL-10, IL-12, IL-13, IL-18, IL-1 receptor antagonist, soluble IL-2 receptor, soluble TNF receptor 2, and C-C chemokine ligand (CCL)-2 (also referred to as monocyte chemoattractant protein [MCP]-1) as well as reduced interferon (IFN)-γ.

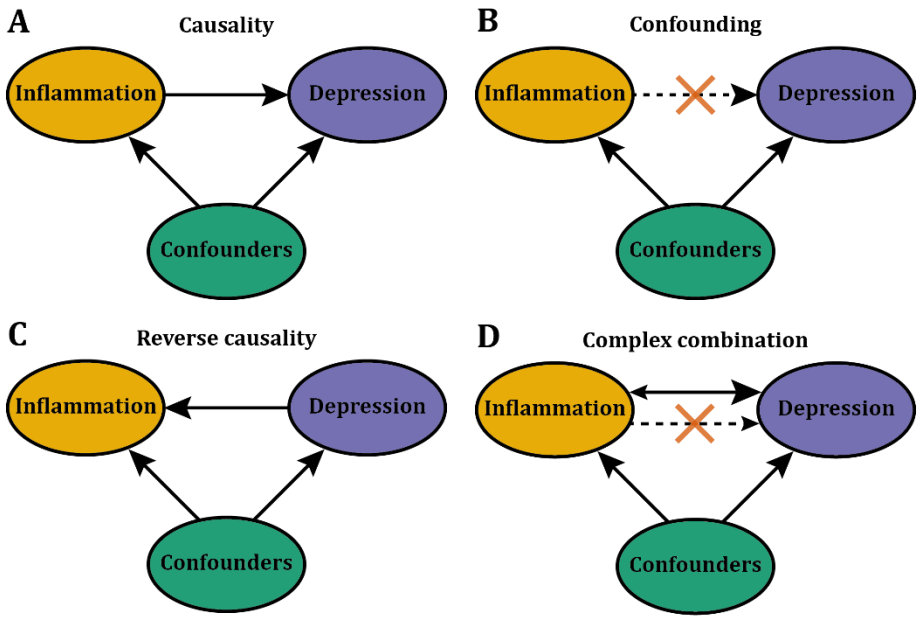

Figure 1. Directed Acyclic Graph (DAG) illustrating differences in the nature of the inflammation-depression association, which could be (A) a true causal association (B) due to confounding, (C) a reversed causal association, or (D) a complex combination such as a partly confounded, truly causal, bidirectional association.

The meta-analyses by Köhler *et al.* (2017) and others provide compelling evidence for case-control differences in pro-inflammatory protein concentrations, but they cannot address key remaining questions on the nature of the inflammation-depression association (see Figure 1). In particular, while the inflammation-depression association may be due

to a true causal effect of systemic inflammation (Fig. 1A), it could also be the result of residual confounding from known or unknown third variables (Fig. 1B), or it may reflect reverse causality, whereby the state of depression itself leads to increased levels of inflammation (Fig. 1C). Adding to these possible models, the inflammation-depression association may also be a complex combination of these models (Fig. 1D); for instance, inflammation may have a true causal effect for depression, but this association may be partly confounded and could be bidirectional.

1.2.2 Evidence from longitudinal studies

Longitudinal studies are one step closer to allowing inferences on causality. They can be used to provide evidence for two of Bradford Hill's viewpoints on causality (Bradford Hill, 1965), namely temporality (i.e., changes in the exposure precede changes in the outcome) and biological gradient (i.e., larger changes in the exposure result in larger changes in the outcome). In one of the first studies of this kind and using data from 2,447 children in the Avon Longitudinal Study of Parents and Children (ALSPAC), Khandaker *et al.* (2014) indeed showed that serum levels of IL-6 at age nine years were longitudinally associated with depressive symptoms at age 18 years in a linear dose-response fashion. Importantly, this association persisted after adjustment for important sociodemographic and lifestyle confounding variables and after adjusting for past psychological and behavioural problems. Evidence from such longitudinal studies has recently been synthesised in the first systematic review and meta-analysis including 38 studies reporting on data from 58,256 participants (Mac Giollabhui, Ng, Ellman, & Alloy, 2020). Results were in favour of a bidirectional association of IL-6 and depression, whereby IL-6 was associated with future depressive symptoms and *vice versa*. Results for CRP were less conclusive with association estimates attenuating in meta-regression and there was no support for longitudinal associations of TNF-α. Overall, these results support bidirectional causal models for selected pro-inflammatory markers such as IL-6 (cf. Fig. 1A & 1C), but residual confounding cannot be fully excluded by longitudinal research, so remains a possibility.

1.2.3 Experimental evidence

To formally test if systemic inflammation exerts a causal effect on depression, evidence from experimental approaches is required that selectively modulate the exposure (here: inflammation), but not potential confounding variables, and evaluate effects of the exposure on the outcome (here: depression). Currently, the evidence from experimental approaches originates from animal studies, experimental medicine studies of immune-modulating drugs and from studies using Mendelian randomisation (MR) analyses.

1.2.3.1 Animal and human studies of immune-modulating drugs

As early as in the late 1980s and early 1990s, experimental studies in animals have demonstrated that activation of the immune system following infections or administration of pro-inflammatory cytokines induces so-called sickness behaviour in animals including loss of interest in activities, social isolation, hypersomnia, and anorexia among others, which mirror symptoms of depression in humans (Dantzer, 2001; Hart, 1988; Kent, Bluthé, Kelley, & Dantzer, 1992). Evidence from human studies has led to similar results as drugs that promote states of systemic inflammation are associated with the emergence of depressive symptoms; for instance, a large literature base has demonstrated that cancer patients treated with pro-inflammatory treatments such as interferon-α or IL-2 develop symptoms consistent with a diagnosis of MDD in up to 50% of patients (Capuron, Ravaud, & Dantzer, 2001; Musselman et al., 2012; Raison & Miller, 2003). A similar pattern of results has been observed in patients with hepatitis C who also are at increased risk of developing depression subsequent to interferon treatment (Udina et al., 2012). These experimental results of increased inflammation leading to sickness behaviour and depressive symptoms also support evolutionary conceptualisations of inflammation and depression, whereby symptoms such as anhedonia, social isolation, and fatigue could foster states of retreat, recovery and protection from pathogens (Miller & Raison, 2016).

Evidence from clinical trials of anti-inflammatory drugs has also been accumulating providing the flipside perspective on potential causality of the inflammation-depression association. Non-steroidal anti-inflammatory drugs (NSAIDs) and newer anti-cytokine drugs such as monoclonal antibodies (mAbs) are commonly used to treat illnesses associated with chronic inflammation such as rheumatoid arthritis. As depressive symptoms are often assessed as potential side effects in RCTs of these drugs for chronic inflammatory conditions, this offers the opportunity to evaluate the treatment potential of immune-modulating drugs, and thus their causal effect, on depressive symptoms.

The effectiveness of anti-inflammatory drugs for treatment of depressive symptoms in chronic inflammatory illnesses has been summarised in multiple systematic reviews and meta-analyses (Kappelmann, Lewis, Dantzer, Jones, & Khandaker, 2018; Köhler-Forsberg et al., 2019; Köhler et al., 2014; Wittenberg et al., 2020). In the first of these studies, Köhler *and colleagues* (2014) have synthesised evidence from RCTs using NSAIDs and anti-cytokine drugs, which showed evidence for small-to-moderate benefits of these drugs in treating depressive symptoms compared to placebo arms (standardised mean difference [SMD]= 0.34, 95% CI: 0.11-0.57) based on data from 5,447 participants. Subsequent meta-analyses have resulted in similar results with SMD of 0.40 (95% CI: 0.22–0.59) in a meta-analysis of anti-cytokine drugs based on 2,370 participants (Kappelmann et al.,

2018), with SMD of 0.29 (95% CI: 0.12-0.45) in a meta-analysis of industry anti-cytokine data from 10,743 participants (Wittenberg et al., 2020), and SMD of 0.49 (95% CI: 0.33-0.64) in an update of the initial meta-analysis on multiple types of anti-inflammatory drugs (Köhler-Forsberg et al., 2019). Importantly, evidence from meta-regression and stratified analyses in two of these meta-analyses suggested that the benefits of the anti-inflammatory drugs were not fully explained by improvements in physical illness symptoms of the condition under investigation (Kappelmann et al., 2018; Wittenberg et al., 2020). This suggests that anti-inflammatory drugs have anti-depressant effects that are (at least partly) independent from the pathophysiology of the autoimmune condition under investigation. Taken together, these findings highlight a potential causal role of inflammation for depressive symptoms in the context of physical illnesses.

Outside the context of physical illnesses, however, these RCTs cannot ascertain if effectiveness of anti-inflammatory drugs persists for psychiatric patients with MDD. In MDD, only few studies with small sample sizes have been conducted so far. Regarding cytokine inhibitors, the first RCT investigated the effectiveness of the anti-TNF-α mAb infliximab versus placebo for 40 patients with MDD and results did not show any overall benefit of infliximab versus placebo (Raison et al., 2013). In post-hoc analyses, however, the authors showed that infliximab resulted in greater response rates for patients with CRP>5mg/L as compared to those with CRP<5mg/L. In a subsequent RCT, McIntyre *and colleagues* (2019) have replicated the study design of the original infliximab RCT in an investigation of bipolar depression. While the authors did not find that baseline CRP levels moderated the drug's effectiveness, self-reported levels of childhood maltreatment, which are also associated with inflammation (Baumeister, Akhtar, Ciufolini, Pariante, & Mondelli, 2016), predicted treatment response. Lastly, there is also evidence that baseline CRP levels moderated the effectiveness of minocycline, a tetracycline antibiotic with anti-inflammatory properties, as adjunct to monoaminergic treatment (Nettis et al., 2021).

1.2.3.2 Evidence from Mendelian randomisation studies

MR is a genetic approach to untangling causality, which takes advantage of Mendel's law that genetic variants are inherited randomly from parents to offspring (Hemani, Zheng, et al., 2018). In this way MR is similar to a clinical trial (Fig. 2A) and allows for valid tests of causality if three major assumptions are met. These assumptions state that (i) the genetic variants or single nucleotide polymorphisms (SNPs) are associated with the exposure, (ii) these SNPs are not directly associated with the outcome if the exposure is controlled for, and (iii) the SNPs have no direct effect on potential confounding variables that could mediate effects on the outcome (Fig. 2B). Regarding the effect of inflammation on depression, there have been two prior MR studies investigating evidence for potential

causality. The first study was conducted by Wium-Andersen *and colleagues* (2014) who analysed data on serum CRP, genotypes of four SNPs located in the *CRP* gene, and information on hospitalisation or death with depression in two Danish general population samples including 78,809 individuals. While the authors observed associations of serum CRP concentrations with depression, there was no association between genetically predicted CRP and genetically predicted hospitalisation or death with depression from MR analysis (OR=0.79, 95% CI: 0.51-1.22). The second study was conducted by Khandaker *and colleagues* (2020) who used data from 367,703 unrelated individuals from the UK Biobank study with 4% of the sample qualifying for probable moderate or severe lifetime depression. Selecting four SNPs in the *CRP* gene associated with serum CRP concentrations and three SNPs in the *IL6R* gene associated with serum IL-6 concentrations, the authors observed potential causal associations of increased genetically predicted CRP (OR=1.35, 95% CI: 1.12–1.62) and of increased genetically predicted IL-6 (OR=1.18, 95% CI: 1.07–1.29) with probable lifetime depression. Beyond CRP and IL-6, Khandaker *et al.* (2020) also observed a MR association of genetically predicted increases in triglycerides with probable lifetime depression (OR=1.18, 95% CI: 1.09–1.27) suggesting that a combination of these immuno-metabolic risk factors may causally contribute to depression and cardiovascular disease, which commonly occur comorbidly (Gold et al., 2020).

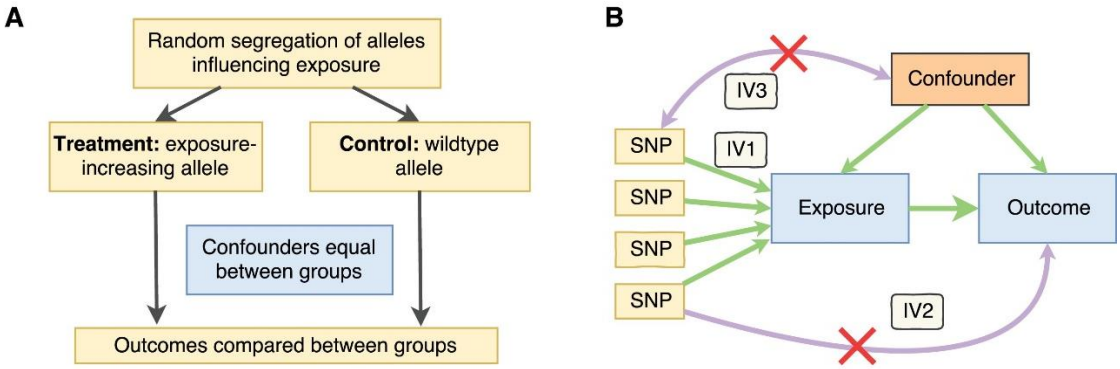

Figure 2. This figure illustrates the (A) principles of Mendelian randomisation (MR) studies and their complementarity to clinical trials and (B) the assumptions underlying MR analyses, which are abbreviated with IV (instrumental variable). This figure has been reproduced under Creative Commons Attribution License from Hemani *et al.* (2018).

In sum, these results provide mixed support for potential causal effects of inflammation in depression. While findings of immune-modulating drugs in autoimmune conditions and cancer support a causal role of inflammation in depression and depression-like behaviour in animals, results in MDD suggest anti-inflammatory treatments may not exhibit overall effectiveness and could instead be specific to a potential subgroup of patients with evidence of low-grade inflammation. Current research efforts are trying to characterise

these subgroups in terms of immunological, metabolic, and clinical complexity, which is also the focus of the current thesis.

1.3 Complexity of the inflammation-depression association

1.3.1 Immunological complexity

Regarding immunological complexity, most studies to date have investigated depression in the context of associations with CRP. CRP is a broad inflammatory marker and used throughout medicine to index inflammation as a substrate of the acute phase response (Cray, Zaias, & Altman, 2009). However, CRP has complex pro- and anti-inflammatory roles, so investigating CRP could be problematic as a uniform index of systemic low-grade inflammation (Del Giudice & Gangestad, 2018). Cytokines could represent more specific markers as they have more circumscribed immunological functions such as differentiation of naïve T helper (T_H) cells into specific T_H cell lines (e.g., T_H1, T_H2, T_H17 or T_{reg} cells), stimulation of antibody production, or induction of lymphocyte chemotaxis (Himmerich, Patsalos, Lichtblau, Ibrahim, & Dalton, 2019). Cytokines also constitute plausible mediators of neurobiological functioning; for instance, IL-1β, IL-6, and TNF-α have been implicated in central plasticity and learning processes (McAfoose & Baune, 2009).

As outlined in section 1.2.1, case-control studies have highlighted the most consistent associations of depression with IL-6 and TNF-α among others (Köhler et al., 2017). These cytokines have also been implicated in chronic inflammatory illnesses such as rheumatoid arthritis and psoriasis and they constitute treatment targets in these illnesses (Kappelmann et al., 2018; Wittenberg et al., 2020). It remains unclear, however, what signalling pathways underlie their potential effects in depression. For example, IL-6 can bind to membrane-bound IL-6 receptors (IL-6R) that are present on few different cell types such as certain lymphocytes and hepatocytes. Together with glycoprotein 130 (gp130) this can induce so-called IL-6 classic signalling with downstream pro- and anti-inflammatory effects including the acute-phase response and CRP production (see Figure 3). However, IL-6 can also induce signalling by binding with soluble IL-6Rs, which together with gp130 initiates the largely pro-inflammatory IL-6 trans-signalling on cells that do not naturally express IL-6Rs (Hunter & Jones, 2015). Importantly, it is currently unclear which of these pathways (if any) are implicated in depression and currently approved anti-IL-6 drugs such as tocilizumab do not selectively block specific IL-6 pathways, which may lead to unnecessary drug side effects.

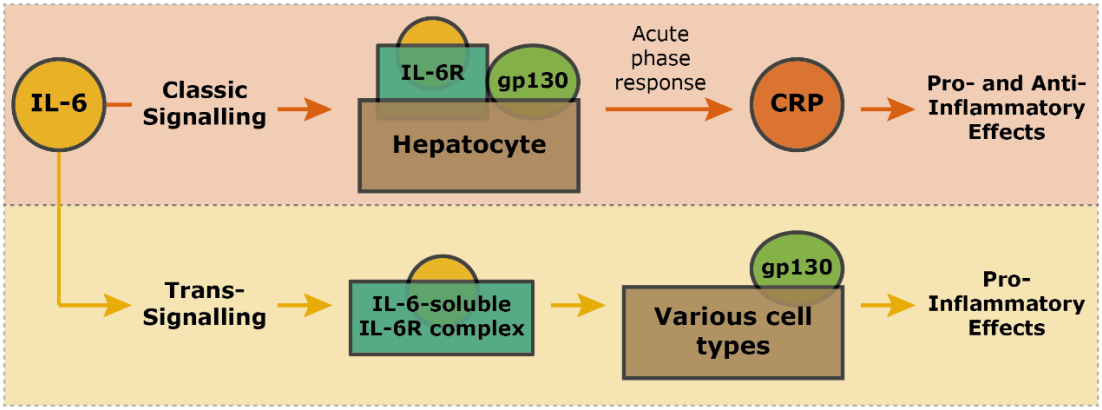

Figure 3. Illustration of current understanding of IL-6 biology; see Hunter and Jones (2015) for a review. Figure reproduced and adapted from Ye and Kappelmann *et al.* (2021); see Appendix B.

Beyond the unclarities around IL-6 signalling pathway involvement, other mechanistic unclarities remain as well. For instance, there is substantial immunological complexity in the CNS as (i) cytokine receptors are present on multiple cell types including astrocytes, microglia, and neurons, (ii) these cells interact locally following cytokine signalling leading to alterations in monoaminergic and glutamatergic neurotransmission, and (iii) inflammation leads to systems-level alterations in communication between different brain regions such as between prefrontal cortex, anterior cingulate, insula, hippocampus, and amygdala (Miller & Raison, 2016). These examples show that future research needs to clarify which cytokines are selectively implicated in depression and which signalling pathways mediate their risk-increasing effects peripherally and centrally. In turn, this information can help to potentially pinpoint existing drugs with anti-depressant potential and/or it could pave the way for the development of new therapeutics that are specific for potential pathways such as IL-6 trans-signalling.

1.3.2 Metabolic complexity

Metabolic complexity provides a potential additional contributor to the association of inflammation and depression as multiple plausible metabolic pathways could influence depression and specific depressive symptoms dependently and independently from inflammation.

Most importantly, there are intricate bidirectional associations between metabolic dysregulation and systemic inflammation. Infiltrating macrophages in white adipose tissue produce pro-inflammatory cytokines (Osborn & Olefsky, 2012). Additionally, lipids, ceramides, and reactive oxygen species can activate pattern recognition receptors (PRRs) for pathogen-associated and damage-associated molecular patterns (PAMPs & DAMPs) in subcutaneous or visceral adipose tissue. This initiates formation of NOD-like receptor

(NLPR) 3 inflammasomes in the cytoplasm that are important regulators of innate immunity following infections as they initiate cell death through pyroptosis (Sharma & Kanneganti, 2021). In the absence of infection, however, NLPR3 inflammasomes can promote aggravation of inflammation via IL-1β and IL-18 release and disruption of insulin signalling, which can lead to a vicious cycle of adiposity and inflammation (Sharma & Kanneganti, 2021). NLPR3 inflammasomes can also promote glucocorticoid receptor (GR) cleavage and thereby reinforce hypothalamus-pituitary-adrenal (HPA) axis overactivation (Milaneschi, Simmons, van Rossum, & Penninx, 2019). Lastly, pro-inflammatory signalling can also promote insulin resistance and reduce glucose uptake through direct effects on pancreatic β-cells (Branchi et al., 2020).

Metabolic dysregulation can also lead to effects on the brain independent of inflammation. MDD and BMI GWA studies have pointed out jointly implicated genes such as neuronal growth regulator 1 (NEGR1), which affects synaptic plasticity mechanisms in brain regions relevant for mood and appetite regulation (Milaneschi et al., 2019). Partitioned heritability studies have further suggested that heritability for increased BMI is related to polygenic contributions of central nervous system (CNS) cells (Finucane et al., 2015) and expression of glutamatergic neurons in particular (Finucane et al., 2018). Mechanistically, leptin signalling could be one of the drivers of altered energy homeostasis in these brain systems. Leptin is an anorexigenic molecule that usually confers satiety signals after a meal (Cui, López, & Rahmouni, 2017). In obesity, however, states of leptin resistance can develop due to ineffective leptin transport across the BBB or dysfunctional leptin receptors (Milaneschi et al., 2019). This can alter hypothalamic signalling to reward circuits relevant for wanting and liking aspects of food, brainstem nuclei responsible for autonomic nervous system functioning, and executive control networks, which ultimately disinhibits eating behaviour and favours energy intake over expenditure (Cui et al., 2017; Richard, 2015).

1.3.3 Clinical complexity

Regarding clinical complexity, studies have suggested early on that symptoms falling under the "sickness syndrome" umbrella (e.g., fatigue, sleeping problems, changes in appetite) may be most relevant in a state of inflammation as the state of sickness itself phenotypically aligns with these depressive symptoms (Dantzer et al., 2008). This hypothesis has gained an increasing amount of support from cross-sectional general population-based and case-control studies that have mainly found associations of inflammatory markers such as CRP with fatigue (Jokela, Virtanen, Batty, & Kivimäki, 2016; White, Kivimäki, Jokela, & Batty, 2017), sleeping problems (Jokela et al., 2016; White et al., 2017), and changes in appetite (Jokela et al., 2016; Lamers, Milaneschi, de Jonge, Giltay,

& Penninx, 2018; Simmons et al., 2020) while there has been less support for associations with other symptoms such as depressed mood, anhedonia, or other more psychological symptoms such as feelings of guilt (Köhler-Forsberg et al., 2017; White et al., 2017). Changes in appetite in particular has received a lot of attention as increased appetite is a key feature of atypical depression, which is more prevalent among women and has also been specifically associated with certain inflammatory markers (Lamers et al., 2018; Rainville & Hodes, 2019; Simmons et al., 2020). Cross-sectional studies also suggest associations of pro-inflammatory markers with anxiety disorders (Costello, Gould, Abrol, & Howard, 2019), which are highly comorbid with depression (Kendler, 1996), but the association with individual anxiety symptoms has not been investigated in detail. Overall, just like cross-sectional research on composite depression phenotypes, however, these studies cannot ascertain directionality and the influence of confounding factors, that have been variably adjusted for in these studies, remains unclear.

Recently, studies using longitudinal designs and genetic analyses have moved more closely to untangling the symptom-specificity of inflammation. For instance, Chu *and colleagues* (2019) report data from the ALSPAC study that demonstrated longitudinal associations of IL-6 at age 9 years with fatigue, sleeping problems, concentration difficulties and diurnal variation in mood at age 18 years after adjusting for age at initial assessment, sex, ethnicity, paternal occupation, BMI and self-reported infection at time of blood collection. An alternative approach to longitudinal analyses has been the use of polygenic risk scores (PRS), which use SNP effect sizes from large-scale genome-wide association study (GWAS) summary statistics as weights to calculate a genetic score for predisposition to the same phenotype in a target sample (Choi, Mak, & O'Reilly, 2020). Milaneschi *and colleagues* (2017) have used this approach to show that MDD with increased appetite, but not MDD with decreased appetite, is associated with a CRP PRS. While both of these studies offer further support for longitudinal associations of inflammatory markers with individual symptoms or symptom-based MDD phenotypes, both longitudinal cohort and PRS investigations can still be biased from unmeasured confounding. For instance, PRSs usually consist of a multitude of SNPs across the genome, so they map information from highly pleiotropic pathways underlying their target phenotype. This was particularly evident in the study by Milaneschi *and colleagues* (2017) who observed similar associations of MDD with increased appetite with BMI and CRP PRSs. Since CRP and BMI show moderate genetic correlations (Ligthart et al., 2018), it thus remains unclear if inflammation, metabolic dysregulation or an unmeasured third factor could underlie the observed associations. Understanding the role of potential confounders such as metabolic dysregulation is important, however, since it may point out whether anti-inflammatory approaches or other treatments such as lifestyle interventions targeting metabolic dysregulation could be more promising in depression. Therefore, experimental evidence

such as from clinical trials or MR are needed to evaluate potential symptom-specificity of inflammation further.

A final drawback of prior studies has been a focus on investigating inflammation-depression associations under the "common cause" hypothesis. The common cause hypothesis represents medicine's overarching disease framework, in which symptoms are passive indicators of the underlying disease. According to this framework, for instance, lung cancer *causes* symptoms such as cough or chest pain, but these symptoms should be statistically independent from each other once controlling for the underlying disease. This framework has also been applied to MDD, which would mean associations of inflammation with individual symptoms are mere indicators of associations to the underlying MDD illness phenotype (Figure 4A). Contrary to this framework, however, recent conceptualisations have considered MDD from a so-called complexity perspective. Under this perspective, symptoms are thought to causally interact in their own right rather than being mere passive illness indicators (Borsboom, 2017). This formulation aligns with clinical perceptions, in which a symptom such as fatigue can trigger other symptoms such as hypersomnia or sad mood (Figure 4B). Following this framework, complex symptom interactions could themselves underlie illness expressions, for instance, through self-reinforcing vicious symptom cycles.

If depressive symptoms followed such a complexity framework, this would also have implications for association studies of inflammatory markers and individual symptoms. These studies have usually evaluated associations for each symptom in isolation without controlling for respective other depressive symptoms (e.g., Chu et al., 2019). While this work is important and can point out potential symptom specificity of inflammatory markers, these analyses cannot ascertain if associations to some symptoms are direct or reflect indirect associations via other depressive symptoms. Taking the example model in Figure 4B, bivariate association analyses cannot discern that associations of inflammation with sad mood and sleeping problems are only indirect and mediated via fatigue (Figure 4C). Making such distinctions is important, however, as symptoms that are the direct consequence of inflammation could theoretically be targeted best by treating the inflammation while symptoms that are only indirect consequences of inflammation could also be targeted by interrupting causal effects of the mediating symptom (e.g., preventing sleeping problems by targeting fatigue through behavioural activation). Disentangling such direct and indirect associations requires multivariate approaches that assess partial/unique associations (Figure 4D).

Recently, network analysis techniques have emerged as a novel analytic strategy to assess direct and indirect associations (Epskamp, Borsboom, & Fried, 2018). Thus far, two studies have applied this analysis strategy to evaluate associations of CRP, IL-6, and TNF-

α with individual depressive symptoms (Fried et al., 2019; Moriarity, Horn, Kautz, Haslbeck, & Alloy, 2021; n.b., only Fried et al. have assessed IL-6 and TNF-α). The most consistent associations emerging from these analyses were between serum CRP and changes in appetite and fatigue, suggesting that these symptoms could be directly associated with inflammation. However, both of these studies relied on cross-sectional data, again highlighting the potential for residual confounding and reverse causality.

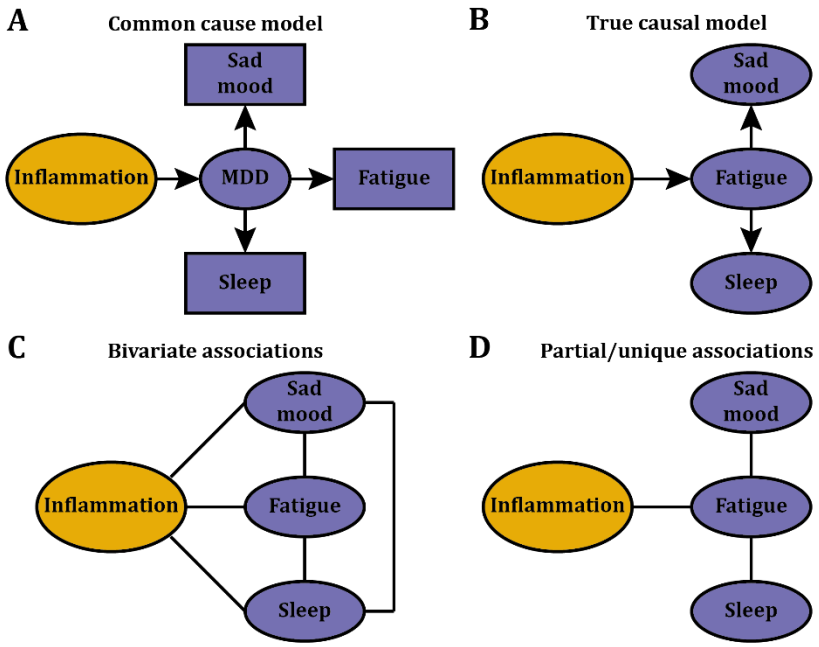

Figure 4. DAGs illustrating the inflammation-depression association under (A) a common cause view, whereby inflammation causes MDD and symptoms are merely passive illness indicators, and under (B) a hypothesised true causal model, in which symptoms are causal agents, so inflammation may cause individual symptoms such as fatigue that then causally influence other symptoms such as sad mood and sleeping problems. Assuming the causal model presented in Figure 4B, association analyses would lead to differences between (C) bivariate and (D) partial/unique associations, which are reflected here as lines/edges.

1.4 Aims and results of this thesis

The aim of this thesis was to advance our understanding on the association of inflammation and depression. To meet this aim, the objective of the first study was to use existing GWAS data sources (i) to quantify potential genetic correlation between inflammation, metabolic dysregulation, and specific depressive symptoms and (ii) to test potential causality of association using MR analysis. Results from genetic correlation and MR analyses showed that CRP exhibits consistent co-heritability across depressive symptoms while the potential causal underlying factors of this co-heritability could be increased BMI for four depressive symptoms and increased IL-6 activity for suicidality. The objective of the

second study was to evaluate direct and indirect associations of specific depressive symptoms and genetic predisposition to higher inflammatory markers and BMI using network analysis in three large general-population-based and patient samples. This showed that the CRP PRS had a consistent direct association with changes in appetite while there were less consistent associations of the CRP PRS with fatigue and anhedonia, the TNF-α PRS with fatigue, and the BMI PRS with changes in appetite and anhedonia. In two further studies using large cohort data from the Netherlands Study of Depression and Anxiety (NESDA) and UK Biobank studies (Appendices A & B), we showed that CRP was associated with depressive rather than with anxiety symptoms, which provided evidence for disorder-specificity. Among depressive symptoms, the most consistent associations emerged with fatigue and sleeping difficulties and MR indicated that these associations could be due to a causal effect of increased IL-6 activity.

Together, the results from this thesis point towards symptom-specificity of the inflammation-depression association and the importance of symptom directionality, they highlight the interwoven nature of inflammation and metabolic factors, and they suggest potential immunological specificity for IL-6 rather than CRP. Each of these intricacies and their potential interpretations is discussed in further detail below and in light of a plausible causal model underlying these findings (see Figure 4). This is then followed by discussion on limitations, future outlook, and conclusion.

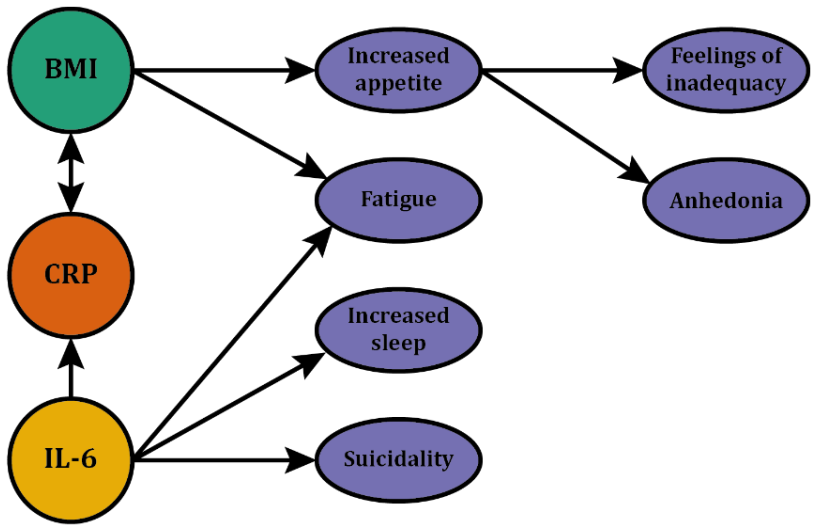

Figure 4. Schematic overview of simplified plausible causal model underlying findings from this thesis and previous literature.

1.5 Refining evidence on symptom-specificity

Although there appears to be consistent co-heritability between CRP and BMI with all depressive symptoms, we observed consistent evidence for symptom-specificity across

the four studies. Serum CRP and IL-6 seemed to be specifically associated with depressive rather than anxiety symptoms (Appendices A & B). Among depressive symptoms, higher serum CRP, polygenic risk for CRP, and serum IL-6 were specifically associated with fatigue and sleeping difficulties (Study 2 & Appendix A). There were also associations of higher serum CRP and polygenic risk for CRP with changes in appetite and specifically with increased appetite, but associations with serum CRP attenuated once controlling for BMI (Study 2 & Appendix A). In contrast, higher serum IL-6 was associated with decreased appetite even after controlling for BMI (Appendix A). MR analyses did not suggest that higher CRP was causally contributing to increased risk for depressive symptoms (Study 1, Appendices A & Appendix B). However, increased IL-6 activity was a potential causal factor for suicidality, fatigue, and sleeping difficulties and we also observed MR associations of increased BMI with depressive symptoms of changes in appetite, fatigue, anhedonia, and feelings of inadequacy (Study 1 & Appendix A).

Taken together, the findings from this thesis most consistently replicate previous findings on changes in appetite, fatigue, and sleeping problems. Regarding changes in appetite, a large body of prior cross-sectional, longitudinal, and network analysis studies using polygenic risk scores and serum inflammatory markers have suggested associations between CRP, IL-6, TNF-a, and BMI with changes in appetite (Fried et al., 2019; Jokela et al., 2016; Lamers et al., 2018; Milaneschi, Lamers, & Penninx, 2021; Milaneschi et al., 2017; Moriarity et al., 2021; Pistis et al., 2021; Simmons et al., 2020). We add to this evidence in Study 2 by showing this is likely to be a direct effect as highlighted by associations with PRSs for BMI and CRP. In Study 1, we further show that the association is likely arising from a causal effect of BMI suggesting that metabolic dysregulation could be a causal factor underlying expression of this symptom. Importantly, studies that assessed increased and decreased appetite separately observed that immuno-metabolic dysregulations were specific for increased appetite (Badini et al., 2020; Lamers et al., 2018; Milaneschi et al., 2016, 2017; Pistis et al., 2021; Simmons et al., 2020) and Milaneschi *et al.* (2021) have recently used the same MR instruments for BMI that were used in Study 1 to show that increased genetically predicted BMI was specifically associated with MDD with increased appetite. This also aligns with divergent association directions in study 2 (i.e., increased BMI PRS associated with decreased appetite in MARS & STAR*D studies and with increased changes in appetite in the UK Biobank sample) and favours a potential causal etiological role of metabolic dysregulation for increased appetite specifically (cf. Figure 4).

Results for fatigue and sleeping difficulties also align with prior studies, where fatigue showed the most consistent associations across the literature with similar reports from PRS, cross-sectional, and longitudinal studies (Badini et al., 2020; Fried et al., 2019; Jokela

et al., 2016; Lamers et al., 2018; Moriarity et al., 2021). From animal research it is well known that sickness triggers behaviours such as lethargy, inactivity, and sleepiness among other symptoms (Hart, 1988) and humans with autoimmune conditions characteristically suffer from fatigue and sleeping problems (Dantzer et al., 2008). Regarding sleeping problems, it is also interesting to note that data from NESDA suggested associations of CRP and IL-6 with sleeping problems arise from increased rather than decreased sleep (Appendix A), which confirms MR results from Study 1, where we did not observe any association between higher CRP/IL-6 with clinical insomnia while there were some indications for associations of IL-6 activity with the composite symptom of sleeping problems. The specificity to increased sleep (i.e., hypersomnia) rather than decreased sleep again aligns with findings suggesting increased immuno-metabolic dysregulations in atypical depression, in which hypersomnia is one of the key features (Milaneschi, Lamers, Berk, & Penninx, 2020).

Among other symptoms exhibiting associations with immuno-metabolic markers such as feelings of inadequacy with BMI (Study 1), or anhedonia with BMI and CRP (Study 2), our findings for suicidality were most surprising. Clinically, suicidality is a complex behavioural phenotype and directly responsible for a large proportion of MDD-associated mortality, particularly in young and otherwise healthy individuals (Otte et al., 2016; Turecki et al., 2019). Therefore, it was surprising to identify a potential causal role of a druggable molecule such as IL-6 for suicidality. Previous research has suggested that IL-6 is increased in cerebrospinal fluid (CSF) and post-mortem brains of suicide attempters and completers, respectively (Ganança et al., 2016; Lindqvist et al., 2009). Suicidality was also among the subset of symptoms that improved following application of the anti-TNF-α drug infliximab in a previous RCT (Raison et al., 2013). The inconsistency in our serum versus MR findings, however, highlights the need for future research to determine whether our findings on IL-6 and suicidality translate to therapeutic effectiveness. Re-analysis of autoimmune RCT data of anti-inflammatory drugs for their specific effects on suicidality could provide one means to address this outstanding research question.

In sum, findings from the studies included in this thesis suggest that pro-inflammatory proteins such as CRP, IL-6 and TNF-α are associated with a unique profile of depressive symptoms, predominantly including fatigue, sleeping problems, changes in appetite, and suicidality. Granular symptom analyses differentiating between increased/decreased sleep and increased/decreased appetite further reveal that the inflammatory signature may be specific to increases in appetite and sleep. This aligns with findings for atypical depression and suggests that immuno-metabolic factors could be specifically related to a unique depressive symptom profile. Investigation of this profile benefits from in-depth

depressive symptom assessments including granular assessments of diametrically opposing symptoms such as changes in appetite and sleep.

1.6 The role of metabolic dysregulation

The inflammation-dependent and -independent metabolic pathways outlined in section 1.3.2 provide interesting clues for the interpretation of findings in this thesis as they suggest varying contributions of metabolic and immunological signatures across depressive symptoms (see also Figure 4).

Specifically, MR analyses in Study 1 highlighted that higher BMI was potentially causally associated with anhedonia, fatigue, changes in appetite, and feelings of inadequacy. From these four symptoms, we only observed a potential causal effect of IL-6 activity on fatigue (Appendix A). At the same time, network analyses in Study 2 indicated that particularly the association of the BMI PRS with changes in appetite suggested a consistent direct association (the BMI PRS-anhedonia association showed inconsistent valence). There could be two plausible explanations for these findings. First, they could indicate that the causal effect of higher BMI on changes in appetite could be mediated by processes independent from the immune system and, since other studies have suggested the association is specific to increased appetite (Milaneschi et al., 2021; Pistis et al., 2021), leptin could be a promising mediator. Specifically, higher BMI could result in leptin resistance, central alteration of homeostatic and reward processes, and consequent disinhibition of eating behaviour. Second, metabolic dysregulation could act via immunological mechanisms other than the IL-6/IL-6R pathway and here the NLP3 inflammasome and downstream IL-1β and IL-18 signalling could be promising targets for future studies, particularly since IL-1β is known to act centrally through effects on long-term potentiation and depression (McAfoose & Baune, 2009).

Since MR analyses also suggested a causal effect of BMI on feelings of inadequacy and anhedonia, the same mechanisms could play a role here. For these symptoms, however, it could also be possible that they are a consequence of increased appetite and therefore only exhibit an indirect causal association with BMI. This hypothesis also follows from the more inconsistent evidence for direct associations between the BMI PRS with anhedonia in Study 2 and absence for such direct associations in previous network studies (Fried et al., 2019; Moriarity et al., 2021).

Finally, findings for fatigue suggest potential causal roles of both BMI and IL-6 activity (Study 1 & Appendix A). These associations also seem to be direct and not mediated by other symptoms based on evidence from Study 2 and previous network studies (Fried et al., 2019; Moriarity et al., 2021). Consequently, it is plausible that metabolic dysregulation

and IL-6 could have independent, additive or related mechanisms contributing to the emergence of fatigue. This could include IL-6 release from visceral adipose tissue and downstream central effects on brain processes (discussed next in section 1.7) or IL-6-related leptin resistance and downstream alterations in reward and energy-processes (Milaneschi et al., 2019). Since fatigue is part of the sickness behaviour characteristically described in animals, mechanistic research in animals could help to establish the exact CNS pathways through which adiposity and IL-6 could lead to fatigue.

1.7 Moving towards specific immunological pathways

The combination of findings from the studies included in this thesis points to potential immunological complexity involved in the inflammation-depression association. On the one hand, we found consistent evidence for genetic correlations of higher CRP across depressive symptoms (Study 1), of direct associations of higher polygenic risk for CRP with changes in appetite and fatigue (Study 2), and of higher serum CRP with depressive symptoms overall and specific symptoms of fatigue, sleeping problems, depressed mood, and changes in appetite (Appendices A & B). On the other hand, 1-sample and 2-sample MR analyses suggested that higher genetically predicted CRP was either not associated with MDD and specific depressive symptoms (Study 1) or associated with *decreased* risk for depressive symptoms (Appendix A & B). At the same time, higher genetically predicted IL-6 activity, as indexed using CRP readout, was associated with *increased* risk for depressive symptoms overall and specifically with suicidality, fatigue, and sleeping problems (Study 1, Appendices A & B). These MR findings were surprising because IL-6 is a key driver of CRP response under the IL-6 classic signalling pathway (Hunter & Jones, 2015), so we would expect that IL-6 and CRP affect depression risk in the same direction. Since this is not the case, results are likely incompatible with risk-mediation via IL-6 classic signalling and support a role of IL-6 trans-signalling in depression as outlined in Figure 5. Specifically, this model suggests that associations between higher serum CRP with depression could be the result of broad IL-6 activity resulting in concurrent increases of IL-6 classic and trans-signalling, but with IL-6 trans-signalling mediating risk for depression.

If IL-6 trans-signalling was specifically related to depression in a subgroup of patients with symptoms of fatigue, sleeping problems, and suicidality, this would have important clinical implications. First, it would mean that currently approved anti-IL-6 drugs such as tocilizumab or sirukumab could be effective for the treatment of depression as they block both IL-6 pathways. However, it could also mean that these drugs have unnecessary side effects associated with inhibition of IL-6 classic signalling such as the hypercholesterolaemia and weight gain observed following tocilizumab administration (Scott, 2017;

Waetzig & Rose-John, 2012). Selective anti-IL-6 trans-signalling drugs could therefore represent more promising drug candidates for depression as they avoid side effects related to inhibition of IL-6 classic signalling. Here, drugs mimicking sgp130 have been proposed as key candidates since sgp130 blocks IL-6 trans-signalling under physiological conditions and is unrelated to IL-6 classic signalling pathway activity (Waetzig & Rose-John, 2012). While there are no currently approved sgp130 drugs available, promising results have been reported recently for the sgp130 fusion protein olamkicept for the treatment of active inflammatory bowel disease in a phase II RCT (Schreiber et al., 2021). Therefore, if findings on IL-6 trans-signalling in depression replicate, drugs such as olamkicept could be valuable treatment candidates for some patients with depression.

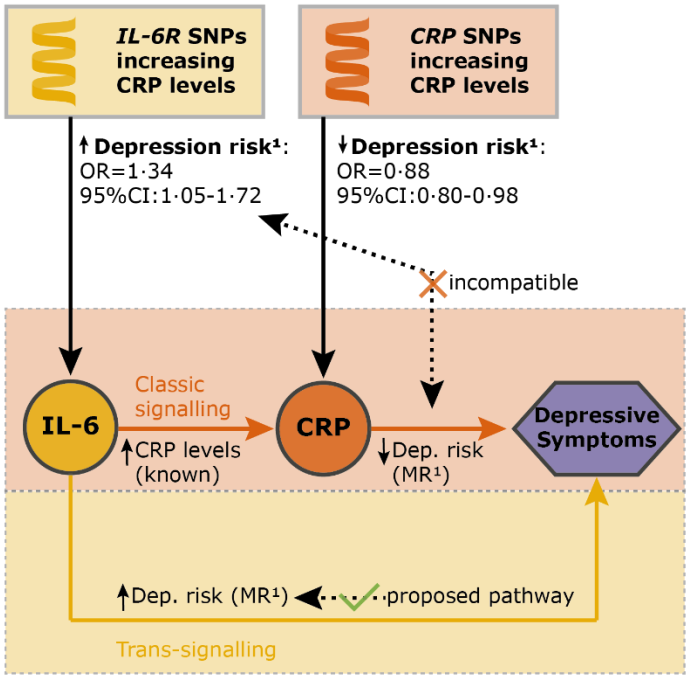

Figure 5. Proposed relationship between MR findings of IL-6 and CRP with depressive symptoms integrated into IL-6 signalling pathways. Figure adapted from Ye and Kappelmann *et al.* (2021). [1]Estimates reflect 2-sample MR results from Appendix B, Table 4.

Using MR techniques, it is difficult to conclusively establish the role of IL-6 classic and trans-signalling since full effects of genetic variants are still mostly unknown and implicated genes code proteins involved in both pathways. For instance, the *IL6R* gene used in MR analyses in this thesis codes both membrane-bound and soluble IL-6Rs. Similarly, genetic variants located in the *IL6ST* gene (which encodes sgp130) and associated with sgp130 concentrations have recently been used in another study as instruments for IL-6 trans-signalling to test potential causality with recurrent depression (Kelly, Smith, & Mezuk, 2021). While findings again pointed to a selective role for IL-6 trans-signalling in

depression, *IL6ST* also encodes the ubiquitously expressed, membrane-bound gp130 necessary for both IL-6 signalling pathways, so findings remain inconclusive due to the pleiotropic roles variants in these genes. Future MR studies may be able to disentangle these pathways further once more fine-grained GWAS summary data become available. For instance, studies could use factorial 1-sample MR designs looking at specific risk-increasing effects of genetic variants associated with greater adamalysin proteases (ADAM) activity of ADAM17 and ADAM10 expression, which promote IL-6R membrane shedding (Hunter & Jones, 2015), in combination with genetic variants associated with higher sgp130 concentrations. Similarly, computational modelling has suggested that the IL-6R to gp130 ratio could constitute an index for IL-6 classic vs trans-signalling (Reeh et al., 2019), so SNPs associated with this ratio could be used as proxies in MR. Generally, however, research would also benefit from studies using alternative approaches to triangulate IL-6 signalling pathway specificity in depression. This could involve analysis of IL-6 pathway-specific transcriptomic/protein indices (e.g., IL-6R to gp130 ratio) as mediators of tocilizumab response in depression RCT data or pharmacological studies testing the anti-IL-6 trans-signalling drug olamkicept in animal models of depression or subsequently in humans.

While this proposed work will help understand the role of IL-6/IL-6R pathways in depression further, future studies also need to investigate other immunological pathways in relation to depression as well as the reactivity of these pathways. For instance, this could involve testing the potential involvement of NLPR3 inflammasomes in depression as noted in section 1.6. Moreover, studies should also investigate the role of other cytokines and individual cell types in depression (Himmerich et al., 2019). Here, investigation of different T helper (T_H) cell lineages could be important. For instance, IL-6 is known to promote greater differentiation of naïve T_H cells towards the T_H17 and away from regulatory T (T_{reg}) cell lineages (Bettelli et al., 2006) and initial work in depression suggests that there is dysregulation of T_H17-T_{reg} cells that could predate excess inflammatory activity in depressed patients (Grosse et al., 2016). Therefore, the T_H17 pathway requires further investigation in depression as well as in relation to specific depressive symptoms.

Finally, the dynamics of the immune response have not been investigated in much detail. In addition to relevance of tonic levels of immune activity, the immune system's primary role is to react in response to the emergence of pathogens. This reactivity is not captured well in studies of genetic or singular serum measures of inflammation and recent work indeed suggests, for instance, that inflammatory indices of lipopolysaccharide (LPS) stimulated serum showed larger associations with depression as compared to a basal inflammation index (van Eeden et al., 2020). Future studies should therefore further explore

associations of depression with dynamic immune alterations, for instance using LPS stimulation of serum samples, direct endotoxin challenge of human participants (Schedlowski, Engler, & Grigoleit, 2014), dynamic immune response assessments (e.g., using saliva measures) following laboratory-based stress tasks (Marsland, Walsh, Lockwood, & John-Henderson, 2017), or simply using multiple measurement time points for inflammatory markers. The latter could also help to assess circadian alterations of these markers that have been reported in autoimmune conditions such as rheumatoid arthritis (Straub & Cutolo, 2007). Taken together, such a focus on system-wide immune changes and innate immune system responsivity could help further elucidate the aetiology of certain depressive symptoms and identify a potential subgroup of patients with depression who may benefit from alternative treatment approaches.

1.8 Limitations and future outlook

Several limitations and requirements for future studies have already been reported in the previous sections. These included calls for more refined symptom measurement including assessments of symptom direction for composite symptoms, investigation of mechanisms underlying associations of higher BMI with symptoms of increased appetite and fatigue, and on the role of individual IL-6 and other immune pathways with depression and symptoms of fatigue, hypersomnia, and suicidality. With regards to the studies of this thesis, however, there are two notable additional limitations and requirements for future research that warrant discussion.

First, associations tested and reported in this thesis rely on observational analyses and experimental animal/human work is needed to fully establish causality between immuno-metabolic markers and symptoms of depression. Cross-sectional and longitudinal studies can be biased from measured and unmeasured residual confounding factors as noted multiple times in this thesis. MR analytic strategies have been developed to circumvent these limitations (Lawlor, Harbord, Sterne, Timpson, & Davey Smith, 2008), but they still rely on three major assumptions. Most importantly, genetic variants may affect the outcome through pathways other than the exposure, which is termed horizontal pleiotropy and which affects genetic correlation and PRS analyses to an even larger extent. In MR, several sensitivity analyses exist to test evidence for horizontal pleiotropy such as MR Egger analysis or Cochran's Q statistic (Hemani, Bowden, & Davey Smith, 2018), which were used in studies of this thesis. However, as long as the functional role of individual SNPs used as genetic instruments is not fully established, horizontal pleiotropy cannot be fully ruled out as alternative explanation. Therefore, triangulation of evidence has been recommended (Lawlor, Tilling, & Davey Smith, 2017; Ohlsson & Kendler, 2020)

and this should ideally involve experimental approaches in animals and humans to confirm/falsify results from this thesis.

Second, studies need to investigate other populations and patient groups to test generalisability of findings and their applicability to certain patient groups. Studies in the present thesis focused on four different samples including UK Biobank, MARS, STAR*D, and NESDA studies. All of these studies are from so-called WEIRD (Western Educated Industrialised Rich Democratic) populations that are not representative of the world's overall population or the world's population of patients suffering from depression (Henrich, Heine, & Norenzayan, 2010). This issue is further aggravated for genetic association analyses in this thesis, which were restricted to individuals of European descent, so future studies need to replicate findings from this thesis in non-WEIRD populations. Genetic association analyses also focused on depression in the context of the UK Biobank general population sample. While evidence consistent with the view of depression as a continuum lends support for this type of investigation (Flett, Vredenburg, & Krames, 1997), qualitative differences between MDD patients as compared to depressive symptoms in non-clinical individuals are also plausible. Additionally, the association of inflammatory markers with depression could be particularly relevant for patients with certain psychiatric or physical illness comorbidities. Therefore, future studies are needed to replicate findings in patients with depression and to explore if associations of inflammatory markers with depressive symptoms in patients are specific for patients with particular psychiatric or physical comorbidities.

1.9 Conclusion

The studies presented as part of this thesis aimed to further untangle the association of inflammation and depression by investigating if associations are specific for certain inflammatory markers, specific for certain depressive symptoms, whether they replicate on the genetic level with regards to co-heritability, and whether they are direct or indirect. Taken together, the evidence from this thesis in combination with prior studies supports a proposed model in which higher BMI is causally associated with increased appetite and fatigue, and increased appetite could have corollary effects on feelings of inadequacy and anhedonia. At the same time, increased IL-6 activity could have causal associations with fatigue, hypersomnia, and suicidality. Certain mechanistic explanations for these pathways have been proposed in this thesis such as leptin resistance or NLPR3 inflammasome activity underlying associations of BMI with increased appetite or IL-6 trans-signalling being responsible for associations of IL-6 with fatigue, hypersomnia, and suicidality. These mechanisms need to be explored in future work using more fine-

grained GWAS data sources and experimental animal/human studies to triangulate evidence for causality. This work should also address limitations of the studies in this thesis such as reliance on WEIRD samples and unclarity on the functional role of individual SNPs used in MR analysis. Thereby, future work could help elucidate and define a potential immuno-metabolic subgroup of depressed patients that could benefit from alternative treatments such as anti-inflammatory drugs or lifestyle interventions leading to a more personalised approach to the treatment of depression.

2. Dissecting the association between inflammation, metabolic dysregulation, and specific depressive symptoms

2.1 Summary

The first study had two major objectives to identify which depressive symptoms are plausible candidates for an inflammation-related aetiology. The first objective was to quantify genetic correlations between CRP, as a broad inflammatory index, and BMI, as an index of metabolic dysregulation, with specific depressive symptoms. The second objective was to evaluate potential causality of CRP, IL-6 activity, and BMI for these specific symptoms. To meet these objectives, we used summary data from available GWA studies on CRP, BMI, specific depressive symptoms, and MDD. We applied linkage disequilibrium score regression to infer SNP-based heritability for these traits and, applying this method to GWAS data from two phenotypes concurrently, to infer SNP-based co-heritability (also termed genetic correlation). Next, we created genetic proxy instruments for CRP levels, IL-6 signalling, and BMI from these GWAS data sources by selecting SNPs associated with higher CRP, IL-6 activity, soluble IL-6Rs, and BMI located around genetic loci coding for these proteins (i.e., SNPs in the *CRP* gene associated with CRP levels & SNPs in *IL6R* gene associated with CRP or sIL-6R levels) or throughout the genome for BMI. Extracting association estimates of the same SNPs with depressive symptom and MDD outcome phenotypes, we then performed inverse-variance weighted (IVW) MR analysis to test for potential causality of effects. We also performed sensitivity analyses using alternative genetic instruments, weighted median MR and MR Egger analysis, and tested heterogeneity of effects to evaluate the potential for horizontal pleiotropy (i.e., effect mediation via pathways other than via the exposure).

Results showed robust genetic correlations of CRP with all depressive symptoms and with the overall MDD phenotype. Similar genetic correlations of these symptoms were present with BMI. Contrary to this, MR analysis indicated that genetic correlations of CRP with depressive symptoms were unlikely to be due to a causal effect of CRP. Yet, we observed a consistent association of increased genetically predicted IL-6 activity with suicidality across MR instruments and sensitivity analyses. We also observed consistent associations of increased genetically predicted BMI with four depressive symptoms, namely anhedonia, fatigue, changes in appetite and feelings of inadequacy.

These results provide evidence for genetic co-heritability between immuno-metabolic markers and specific depressive symptoms. They also indicate that this co-heritability could be due to confounding for some depressive symptoms while increased IL-6 activity could be a causal factor underlying suicidality and increased BMI could be a causal factor

underlying anhedonia, fatigue, changes in appetite, and feelings of inadequacy. These findings provide a more refined characterisation of a potential immuno-metabolic depression phenotype and could open up the opportunity to test new treatments such as anti-IL-6 drugs for suicidality.

2.2 Contributions and reference

The study "Dissecting the Association Between Inflammation, Metabolic Dysregulation, and Specific Depressive Symptoms: A Genetic Correlation and 2-Sample Mendelian Randomization Study" was published in *JAMA Psychiatry* in October, 2020. NK, MKG, GMK, and EBB were responsible for concept and design; NK, JA, MKG, DC, NR, SL, GMK for acquisition, analysis, or interpretation of data; NK, JA, MKG, NR, GMK for statistical analysis; NK for drafting of manuscript; SL, GMK for administrative, technical, or material support; NK, JA, MKG, DC, NR, SL, GMK, EBB for critical revision of content; and JA, DC, GMK, and EBB for supervision.

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JAMA Psychiatry | Original Investigation

Dissecting the Association Between Inflammation, Metabolic Dysregulation, and Specific Depressive Symptoms
A Genetic Correlation and 2-Sample Mendelian Randomization Study

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IMPORTANCE Observational studies highlight associations of C-reactive protein (CRP), a general marker of inflammation, and interleukin 6 (IL-6), a cytokine-stimulating CRP production, with individual depressive symptoms. However, it is unclear whether inflammatory activity is associated with individual depressive symptoms and to what extent metabolic dysregulation underlies the reported associations.

OBJECTIVE To explore the genetic overlap and associations between inflammatory activity, metabolic dysregulation, and individual depressive symptoms.

GWAS DATA SOURCES Genome-wide association study (GWAS) summary data of European individuals, including the following: CRP levels (204 402 individuals); 9 individual depressive symptoms (3 of which did not differentiate between underlying diametrically opposite symptoms [eg, insomnia and hypersomnia]) as measured with the Patient Health Questionnaire 9 (up to 117 907 individuals); summary statistics for major depression, including and excluding UK Biobank participants, resulting in sample sizes of 500 199 and up to 230 214 individuals, respectively; insomnia (up to 386 533 individuals); body mass index (BMI) (up to 322 154 individuals); and height (up to 253 280 individuals).

DESIGN In this genetic correlation and 2-sample mendelian randomization (MR) study, linkage disequilibrium score (LDSC) regression was applied to infer single-nucleotide variant–based heritability and genetic correlation estimates. Two-sample MR tested potential causal associations of genetic variants associated with CRP levels, IL-6 signaling, and BMI with depressive symptoms. The study dates were November 2019 to April 2020.

RESULTS Based on large GWAS data sources, genetic correlation analyses revealed consistent false discovery rate (FDR)–controlled associations (genetic correlation range, 0.152-0.362; FDR $P = .006$ to $P < .001$) between CRP levels and depressive symptoms that were similar in size to genetic correlations of BMI with depressive symptoms. Two-sample MR analyses suggested that genetic upregulation of IL-6 signaling was associated with suicidality (estimate [SE], 0.035 [0.010]; FDR plus Bonferroni correction $P = .01$), a finding that remained stable across statistical models and sensitivity analyses using alternative instrument selection strategies. Mendelian randomization analyses did not consistently show associations of higher CRP levels or IL-6 signaling with other depressive symptoms, but higher BMI was associated with anhedonia, tiredness, changes in appetite, and feelings of inadequacy.

CONCLUSIONS AND RELEVANCE This study reports coheritability between CRP levels and individual depressive symptoms, which may result from the potentially causal association of metabolic dysregulation with anhedonia, tiredness, changes in appetite, and feelings of inadequacy. The study also found that IL-6 signaling is associated with suicidality. These findings may have clinical implications, highlighting the potential of anti-inflammatory approaches, especially IL-6 blockade, as a putative strategy for suicide prevention.

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Supplemental content

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Accumulating evidence implicates the immune system in the pathogenesis of major depression (MD).[1] Low-grade inflammation, as indicated by higher (>0.3 mg/dL [>3 mg/L]) C-reactive protein (CRP) levels, is present in about one-quarter of patients with MD and longitudinally predicts occurrence of depressive symptoms.[2,3] Results of studies[4-9] have suggested specificity of the association of inflammation and depression to a subset of depressive symptoms. C-reactive protein and the proinflammatory cytokine interleukin 6 (IL-6), an upstream stimulator of CRP production, have been reported to be associated with increased appetite, sleep problems, loss of energy, diurnal variation in mood, and concentration difficulties. However, findings vary with regard to which inflammatory markers and depressive symptoms were assessed and which findings were replicated.[6] There is also debate on the robustness of the reported associations after adjustment for metabolic traits, such as body mass index (BMI), which attenuates inflammation-symptom associations.[6] Such attenuation corresponds to recent suggestions of a combined immune-metabolic subtype of depression[10] and warrants further research to disentangle immune from metabolic associations with depressive symptoms.

Associations of inflammation with specific depressive symptom profiles may be clinically relevant. Research suggests that anti-inflammatory drugs may improve depressive symptoms in patients with chronic inflammatory physical illness independent of improvements in physical illness.[11-14] In MD, it has been reported that immunotherapies may be helpful for those patients with evidence of low-grade inflammation or inflammation-associated risk factors.[15,16] Informed by these reports, several ongoing randomized clinical trials (RCTs) are selecting patients with evidence of elevated levels of inflammatory proteins or based on neuroimaging markers of inflammation or inflammation-related symptoms (eg, work by Khandaker et al[17] and 2 other clinical trials[18,19]). Therefore, identification of depressive symptoms that are associated with inflammation is key information that may aid patient selection in future RCTs.

Genetic approaches and increasing availability of genome-wide association study (GWAS) data may enable more fine-grained dissection of inflammation–depressive symptom associations. Genome-wide association studies have highlighted a polygenic architecture underlying both MD and serum CRP levels with many single-nucleotide variants (SNVs) exhibiting small associations.[20,21] Such polygenic associations can be summarized using polygenic risk scores (PRSs),[22] which sum the presence of risk alleles in individuals to create a single score. Milaneschi and colleagues[23,24] have reported that PRSs for both increased CRP levels and BMI are associated with symptom profiles characteristic of atypical (increased appetite or weight) but not typical (decreased appetite or weight) MD. However, it remains unclear from these analyses if symptoms other than changes in appetite or weight underlie the CRP-atypical MD association and whether this association is potentially causal or arising from metabolic factors.

Linkage disequilibrium score (LDSC) regression[25,26] and mendelian randomization (MR)[27] analyses could further dissect the associations between inflammation, metabolic factors, and depressive symptoms. Linkage disequilibrium score regression allows assessment of SNV-based phenotype heritability and coheritability between 2 traits. Mendelian randomization analyses enable an assessment of potential causal association between 2 traits based on the Mendel law that genetic variants are inherited independently, thus providing a natural RCT.[28,29] Initial studies[21,30,31] using LDSC regression and MR analyses reported mixed findings on associations of inflammatory markers and MD. None of these studies examined whether inflammation was associated with specific depressive symptoms.

Using large-scale GWAS data sources, the present study applied a combination of LDSC regression and MR analyses on measures of inflammation, as indicated by CRP levels and IL-6 signaling or activity, BMI, as an index of metabolic dysregulation, and 9 specific depressive symptoms. We tested 2 hypotheses. First, are the associations between inflammation and specific depressive symptoms underpinned by a common genetic basis (ie, coinherited)? Second, is inflammation potentially causally associated with specific depressive symptoms?

Key Points

Question Do inflammatory pathways share a genetic background with individual depressive symptoms, and do they potentially causally contribute to them?

Findings Based on large genome-wide association study data sources, this genetic correlation and 2-sample mendelian randomization study found genetic overlap between a higher C-reactive protein (CRP) level, a broad marker of inflammation, and 9 depressive symptoms; upregulated interleukin-6 signaling, a major stimulator of CRP, emerged as a potential causal risk factor for suicidality. Body mass index, but not interleukin 6 or CRP, was potentially causally associated with 4 other depressive symptoms.

Meaning Interleukin 6 overactivity could be associated with suicidality; interleukin-6 blockade may be a novel treatment target that warrants future research.

Methods

GWAS Data Sources

This genetic correlation and 2-sample MR study was performed from November 2019 to April 2020. Sample sizes and characteristics of GWAS data sources[20,21,32-39] are listed in **Table 1**. They are described in detail in the eMethods in the Supplement.

Briefly, we included GWAS data sources to maximize sample sizes yet avoid sample overlap. These data sources included the following information: serum CRP levels from 204 402 individuals included in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Inflammation Working Group[21]; depressive symptoms from the Neale laboratory[39]; summary statistics for MD from a subset (ie, excluding 23andMe participants) of 2 prior Psychiatric Genomics Consortium (PGC) reports[20,32] that respectively include and

Table 1. GWAS Data Sources

Phenotype	GWAS data source	Sample size	Study or population	Covariates and exclusions	Objective	Reported genome-wide statistically significant hits
CRP levels	Ligthart et al,[21] 2018	204 402	GWAS meta-analysis of 88 studies of European individuals	Covariates: age, sex, population substructure, relatedness. Exclusions: >4 SD above the mean, autoimmune disease, immunotherapy	Primary exposure (LDSC regression and MR)	48 Independent loci
Depressive symptoms	Neale laboratory,[39] 2020	Up to 117 907[a]	UK Biobank study	Covariates: age, age^2, sex, age by sex, age^2 by sex, 20 principal components	Primary outcome	NA
MD	PGC; Wray et al,[32] 2018	Up to 230 214 (45 396 cases and 97 250 controls)[a]	Meta-analysis of PGC studies without UK Biobank and 23andMe samples	Covariates using RICOPILI[38]: age, sex, principal components	Secondary outcome (LDSC regression and MR) and positive control (LDSC regression)	44 Independent loci
MD[b]	PGC; Howard et al,[20] 2019	500 199 (170 756 Cases and 329 443 controls)	Meta-analysis of PGC studies and UK Biobank without 23andMe samples	Covariates in UK Biobank: age, sex, genotyping array, 8 principal components. Covariates in PGC studies using RICOPILI[38]: age, sex, principal components	Secondary outcome (LDSC regression and MR)	101 Independent loci
BMI	GIANT consortium; Locke et al,[33] 2015	Up to 322 154[a]	Meta-analysis of 80 GWAS data in European adults	Covariates: age, age^2, sex, study-specific covariates (eg, genotype-derived principal components)	Secondary exposure (LDSC regression and MR)	97 Independent loci
Insomnia	Jansen et al,[36] 2019	Up to 386 533[a]	UK Biobank without 23andMe sample	Covariates: age, sex, genotype array, 10 genetic principal components	Secondary outcome (LDSC regression and MR)	202 Independent loci
Height	GIANT consortium; Wood et al,[34] 2014	Up to 253 280[a]	Meta-analysis of 79 GWAS data	Covariates: age, sex, study-specific covariates (eg, genotype-derived principal components)	Negative control (LDSC regression)	423 Independent loci
sIL-6R plasma levels	Rosa et al,[37] 2019; Sun et al,[35] 2018	2994	INTERVAL study in the United Kingdom	Covariates: sex, age, duration between blood draw and processing, 3 principal components	Secondary exposure (MR)	NA

Abbreviations: BMI, body mass index; CRP, C-reactive protein; GIANT, Genetic Investigation of Anthropometric Traits; GWAS, genome-wide association study; LDSC, linkage disequilibrium score; MD, major depression; MR, mendelian randomization; NA, not applicable; PGC, Psychiatric Genomics Consortium; RICOPILI, Rapid Imputation for Consortias Pipeline; sIL-6R, soluble interleukin 6 receptor.

[a] Exact sample sizes vary per depressive symptom phenotype (minimum, 117 177; median, 117 822; maximum, 117 907) and per single-nucleotide variant for MD (Wray et al,[32] 2018) (minimum, 55 795; median, 142 646; maximum, 230 241), BMI (minimum, 50 005; median, 233 524; maximum, 322 154), insomnia (minimum, 366 461; median, 385 989; maximum, 386 533), and height (minimum, 50 003; median, 251 631; maximum, 253 280).

[b] Note that depression was characterized differently among samples in the study by Howard et al,[20] including definitions of broad depression, probable MD, and MD diagnosis ascertained from hospital records.

exclude UK Biobank participants, resulting in final sample sizes of 500 199 and up to 230 214 individuals (details are given in the eMethods in the Supplement); BMI (up to 322 154 individuals) and height (up to 253 280 individuals) from the Genetic Investigation of Anthropometric Traits (GIANT) consortium[33,34]; and soluble IL-6 receptor (sIL-6R) protein levels from the INTERVAL study.[35] Genome-wide association studies on depressive symptoms were based on UK Biobank data as assessed in an online follow-up survey using the self-report Patient Health Questionnaire 9 (PHQ-9) (up to 117 907 individuals).[40,41] The PHQ-9 asks about the presence of 9 depressive symptoms, as defined in the *DSM-IV* (Fourth Edition),[42] over the past 2 weeks (eTable 1 and eTable 2 in the Supplement list symptom descriptions and frequency statistics in the UK Biobank sample). Three PHQ-9 symptoms (sleep problems, changes in appetite, and psychomotor changes) do not differentiate between underlying diametrically opposite symptoms (eg, insomnia and hypersomnia). Although these symptoms are included for comprehensiveness of analyses, we emphasize that any associations specific to one (but not the other) underlying symptom are likely obscured in analyses. We have included GWAS data for insomnia[36] (up to 386 533 individuals) to disentangle associations with sleep problems to some extent but could not identify GWAS data for other underlying symptoms.

All original GWAS investigations were conducted with ethics committee approval. The UK Biobank study received approval from the National Health Service National Research Ethics Service. Written informed consent was obtained from participants.

LDSC Regression Analysis

Linkage disequilibrium score regression regresses SNV GWAS χ^2 statistics for 1 phenotype (to infer SNV-based heritability) or χ^2 statistics cross products for 2 phenotypes (to infer SNV-based coheritability) on LDSCs (ie, the sum of a SNV pairwise squared correlation with other SNVs in a 1cM window[43]). Genetic correlations between 2 phenotypes can be inferred by the regression slope.[25,26]

We used LDSC regression to assess the SNV-based heritability (h^2) of all phenotypes and the genetic correlations of CRP levels, MD, BMI, and height with depressive symptoms. For

Table 2. Genetic Instruments for MR Analyses

Exposure	GWAS data source	SNV *F* statistics[a]	SNV location	No. of SNVs		Prior MR report
				Used in present report[b]	Used in prior MR report	
Main MR analyses						
↑CRP levels	CRP GWAS meta-analysis[21]	Minimum, 32.2; median, 89.7; mean, 256.5; maximum, 1829.1	*CRP* gene (within 300-kb region of GRCh37/hg19 coordinates: chr1:159 382 079-159 984 379)	17	24	Georgakis et al,[51] 2020
↑IL-6 signaling	CRP GWAS meta-analysis[21]	Minimum, 48.6; median, 73.8; mean, 144.5; maximum, 458.2	*IL6R* gene (GRCh37/hg19 coordinates: chr1:154 077 669-154 741 926)	6	7	Georgakis et al,[51] 2020
Additional MR analyses						
↑CRP levels (alternative approach)	CRP GWAS meta-analysis[21]	Minimum, 30.0; median, 50.2; mean, 86.5; maximum, 987.2	Genome-wide	139	NA	NA
↑IL-6 signaling (alternative approach)	sIL-6R plasma-level GWAS[35]	Minimum, 16.8; median, 72.2; mean, 271.4; maximum, 5041.9	Within 250-kb region around *IL6R* gene (GRCh37/hg19 coordinates: chr1:154 077 669-154 741 926)	29	34	Rosa et al,[37] 2019
↑BMI	BMI GWAS meta-analysis of Locke et al,[33] 2015	Minimum, 29.0, median, 39.6; mean, 54.7; maximum, 238.5	Genome-wide	95	NA	NA

Abbreviations: BMI, body mass index; chr1, chromosome 1; CRP, C-reactive protein; GWAS, genome-wide association study; IL-6, interleukin 6; kb, kilobase; MR, mendelian randomization; NA, not applicable; sIL-6R, soluble interleukin 6 receptor; SNV, single-nucleotide variant; ↑, increasing.

[a] *F* statistics were computed using the following approximation: $F = \beta^2 \div SE$.[2,37,52]

[b] Available number of SNVs used is reported here for Patient Health Questionnaire 9 depressive symptom outcome; however, these differ per outcome, and exact numbers are listed in eTable 7 in the Supplement.

genetic correlations with depressive symptoms, MD and height served as positive and negative control variables showing strong and absent associations with depressive symptoms, respectively. European ancestry information from the 1000 Genomes Project was used as the linkage disequilibrium reference panel, aligning with European origin of GWAS samples.[44] We used the Benjamini-Hochberg method[45] to control the false discovery rate (FDR) across PHQ-9 symptoms for each phenotype.

2-Sample Mendelian Randomization Analyses

Genetic Instruments

Mendelian randomization uses genetic variants associated with an exposure as instruments to test for potential causal association of this exposure with an outcome. Genetic instruments were based on functional knowledge of the inflammatory pathway underlying CRP production. C-reactive protein is produced in the liver as a consequence of upstream IL-6 signaling via membrane-bound IL-6 receptors (IL-6Rs) on hepatocytes.[46] The IL-6Rs also exist in soluble form in the plasma (sIL-6Rs), but IL-6–sIL-6R complexes are neutralized under physiological conditions.[46-49] Therefore, lower sIL-6R plasma levels constitute an indirect index of IL-6 signaling.[37,49]

We used genome-wide statistically significant, independent ($R^2 < 0.1$), and strong (*F* statistics >10)[50] genetic instruments for higher CRP levels, IL-6 signaling, and BMI[21,33,35,37,51,52] as summarized in **Table 2**. In the eMethods in the Supplement, we describe details for genetic instrument selection, clumping procedure, comparison with previous work,[31] a functional description of included SNVs, and the number of SNVs across instruments and analyses (eTables 3-7 and eFigure 1 in the Supplement). Briefly, we defined 2 main genetic instruments for upregulated CRP levels and IL-6 signaling using SNVs around *CRP* (GenBank 1401) and *IL6R* (GenBank 3570) genes, respectively, that were associated with CRP levels based on CRP GWAS summary statistics.[21,51]

As alternative approaches and to demarcate associations of inflammatory activity from those of metabolic dysregulation,[53] we defined further genetic instruments for CRP levels, IL-6 signaling, and BMI. We used SNVs associated with CRP levels and BMI throughout the genome and SNVs in the *IL6R* gene associated with sIL-6R plasma levels, which were inversed to reflect an indirect marker of IL-6 signaling.

Statistical Analyses

Two-sample MR analyses were performed using *R*, version 3.6.0 (R Foundation for Statistical Computing) and the TwoSampleMR package.[54,55] Exposure and outcome GWAS summary statistics were harmonized by aligning summary statistics to the forward strand if the forward strand was known or could be inferred. Ambiguous SNVs and SNVs with a noninferable forward strand were excluded from analyses.

We first performed fixed-effects meta-analysis of genetic instruments using inverse-variance weighting (IVW).[56] Standard errors were computed with the Wald estimator and delta weighting to account for uncertainty in genetic association with the exposure.[57] To assess the robustness of our findings, the weighted median MR approach was performed, which provides valid estimates if at least 50% of the MR instrument weights on the exposure are valid.[56,58,59]

To assess horizontal pleiotropy (ie, an association of the genetic instrument with the outcome independent of the exposure), we tested for the presence of statistically significant ($P < .05$) heterogeneity in IVW MR analyses using the Cochran *Q* statistic.[60] We also performed more restrictive MR analyses focusing on SNVs within *CRP* and *IL6R* genes (compare gene

loci in Table 2) using MR-Egger estimation for genetic instruments including SNVs throughout the genome, leave-one-out, and single-SNV MR analyses.[58,61,62] Details are available in the eMethods in the Supplement.

All MR analyses were FDR controlled across PHQ-9 symptoms using the Benjamini-Hochberg method.[45] Because main IVW MR analyses for CRP levels and IL-6 signaling focused on 2 genetic instruments, we also corrected these comparisons with the Bonferroni method.

Availability of Data and Materials

Genome-wide association study data sources are openly available as GWAS summary statistics and by request for CRP levels from the CHARGE Inflammation Working Group.[21] Genetic instrument files and analysis scripts are available on the Open Science Framework.[63]

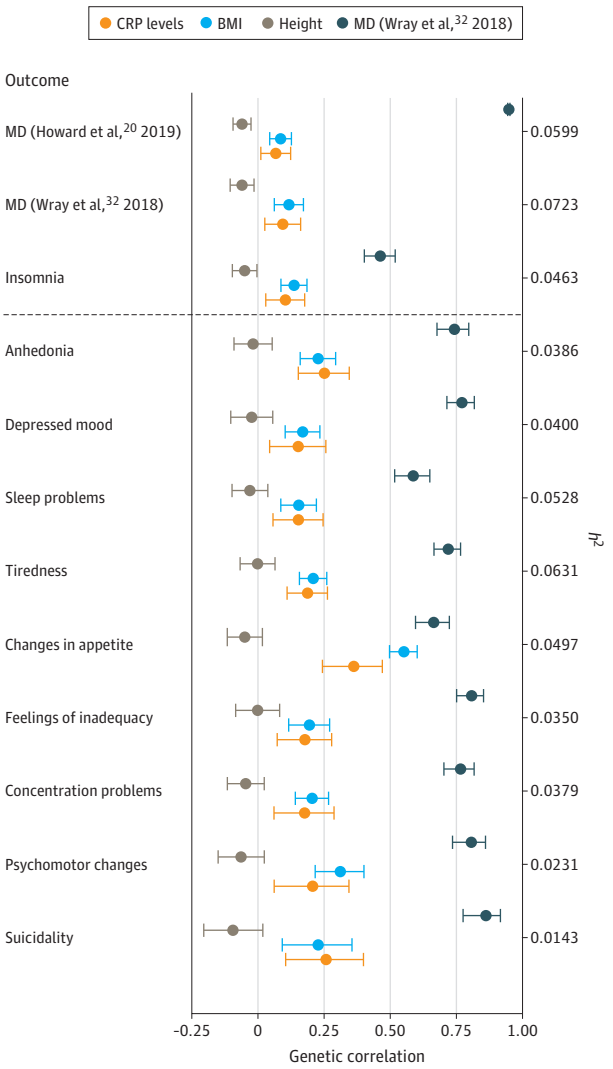
Results

LDSC Regression Analyses

Using LDSC regression, we estimated SNV-based heritability (h^2) and genetic correlations of CRP levels,[21] BMI,[33] MD (based on work by Wray et al[32] as positive control), and height (negative control)[34] with depressive symptoms, MD,[20,32] and insomnia[36] (**Figure 1**). Exact values are listed in eTables 8, 9, and 10 in the Supplement.

The SNV-based heritability was low for depressive symptoms (h^2 range = 0.0143-0.0631), MD (h^2 range = 0.0599-0.0723), and CRP levels (h^2 = 0.0941), whereas BMI (h^2 = 0.1297) and height (h^2 = 0.3120) displayed relatively higher levels. Of note, h^2 for suicidality was slightly below the suggested threshold of $z > 4$ ($h^2 z$ = 3.97), which could reflect a potential unreliability of genetic correlation estimates.[26] There was evidence for genetic correlations of CRP levels with all depressive symptoms after FDR correction (genetic correlation range, 0.152-0.362), with the lowest correlation seen for depressed mood (genetic correlation [SE] = 0.152 [0.056]; FDR P = .006) and the highest for changes in appetite (genetic correlation [SE] = 0.362 [0.067]; FDR P < .001). C-reactive protein levels showed small genetic correlations with MD and insomnia (eTable 10 in the Supplement). Body mass index showed a similar pattern of genetic correlations in that the BMI-depressive symptom estimates were associated with CRP-depressive symptom estimates (Pearson r = 0.89, P = .001; Spearman ρ = 0.92, P = .001).

Figure 1. Single-nucleotide variant (SNV)–Based Heritability and Genetic Correlation Estimates for MD and Depressive Symptoms



The SNV-based heritability coefficients (h^2) for major depression (MD) and depressive symptoms (y-axis) are shown on the z-axis. Sleep problems, changes in appetite, and psychomotor changes reflect composite symptoms, which may obscure associations specific to one but not the other underlying symptom (psychomotor retardation or agitation, increased or decreased weight or appetite, and insomnia or hypersomnia). The error bars indicate 95% CIs, which were calculated using Fisher z transformation. Outcomes below the dashed line are the Patient Health Questionnaire 9 depressive symptoms.

MR Analyses

Mendelian randomization analyses allowed testing of potential causal association between proinflammatory activity and depressive symptoms. **Figure 2** shows MR analyses for CRP levels, IL-6 signaling, and BMI instruments using IVW meta-analysis[20,32] (exact values are listed in eTable 11 in the Supplement).

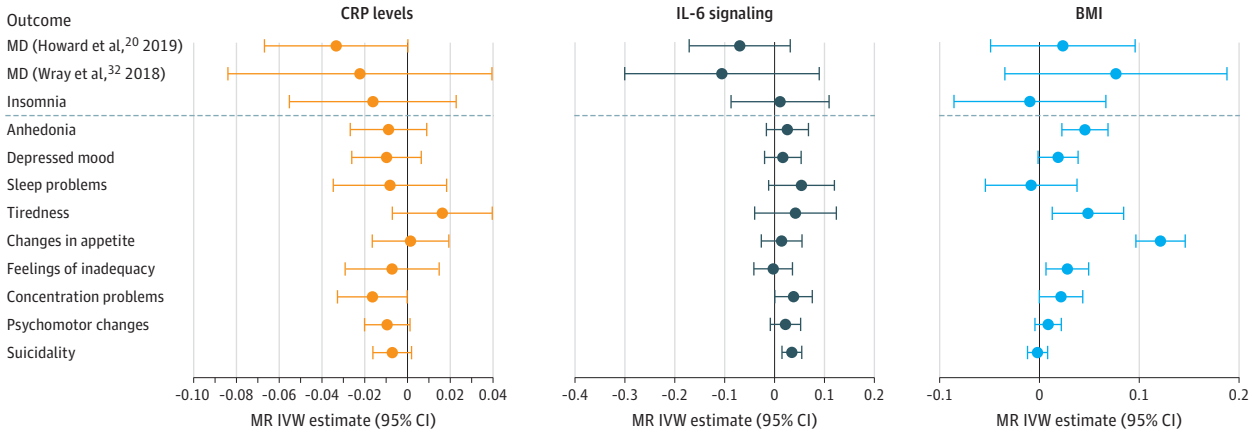
Findings for CRP Levels

Mendelian randomization analyses of the CRP levels instrument did not show evidence for associations with depressive symptoms, MD, or insomnia (eTable 11 and eTable 12 in the Supplement). Using the alternative CRP instrument, there was some evidence for associations of increased CRP levels with tiredness, changes in appetite, and psychomotor changes (**Table 3**),[20,32,45] but none of these associations replicated in weighted median MR (eTable 13 in the Supplement).

Findings for IL-6 Signaling

We observed an association of upregulated IL-6 signaling with suicidality even after conservative FDR and Bonferroni corrections (estimate [SE], 0.035 [0.010]; FDR plus Bonferroni

Figure 2. Mendelian Randomization Inverse-Variance Weighted (IVW) Associations of Genetic Instruments for Upregulated C-Reactive Protein (CRP) Levels, Interleukin 6 (IL-6) Signaling, and Higher Body Mass Index (BMI) With Major Depression (MD) and Depressive Symptoms



Sleep problems, changes in appetite, and psychomotor changes reflect composite symptoms, which may obscure associations specific to one but not the other underlying symptom (psychomotor retardation or agitation, increased or decreased weight or appetite, and insomnia or hypersomnia). The error bars indicate 95% CIs. Outcomes below the dashed line are the Patient Health Questionnaire 9 depressive symptoms.

Table 3. MR IVW Estimates of Alternative Genetic Instruments for Upregulated CRP Levels and IL-6 Signaling

	CRP levels (genome-wide)			IL-6 signaling (indirect)		
Outcome	Estimate (SE)	*P* value	FDR *P* value[a]	Estimate (SE)	*P* value	FDR *P* value[a]
MD (Howard et al,[20] 2019)	−0.021 (0.011)	.06	NA	−0.002 (0.003)	.53	NA
MD (Wray et al,[32] 2018)	0.020 (0.020)	.33	NA	−0.012 (0.007)	.09	NA
Insomnia	−0.010 (0.013)	.42	NA	0.004 (0.003)	.25	NA
Anhedonia	0.002 (0.005)	.64	.64	0.000 (0.002)	.80	.80
Depressed mood	−0.004 (0.005)	.45	.58	0.000 (0.001)	.72	.80
Sleep problems[b]	0.012 (0.008)	.16	.29	0.005 (0.002)	.01[c]	.06
Tiredness	0.021 (0.007)	.002[c]	.02[c]	0.002 (0.002)	.26	.40
Changes in appetite[b]	0.012 (0.006)	.048[c]	.14	0.001 (0.002)	.46	.59
Feelings of inadequacy	−0.003 (0.006)	.63	.64	−0.002 (0.002)	.14	.30
Concentration problems	−0.005 (0.005)	.34	.51	0.003 (0.002)	.06	.19
Psychomotor changes[b]	−0.006 (0.003)	.046[c]	.14	0.001 (0.001)	.24	.40
Suicidality	0.004 (0.003)	.15	.29	0.002 (0.001)	.005[c]	.049[c]

Abbreviations: CRP, C-reactive protein; FDR, false discovery rate; IL-6, interleukin 6; IVW, inverse-variance weighting; MD, major depression; MR, mendelian randomization; NA, not applicable.

[a] *P* values were FDR controlled across depressive symptoms of each outcome using the Benjamini-Hochberg method.[45]

[b] Sleep problems, changes in appetite, and psychomotor changes reflect composite symptoms, which may obscure associations specific to one but not the other underlying symptom.

[c] *P* < .05 for nominal and FDR-controlled statistically significant results.

P = .01) (eFigure 2 in the Supplement), but there were no associations with MD, insomnia, or other depressive symptoms. The IL-6–suicidality association was replicated with the (indirect) IL-6 signaling instrument and across IVW and weighted median MR analyses (main IL-6 signaling weighted median estimate [SE], 0.030 [0.011]; *P* = .006; alternative IL-6 signaling IVW estimate [SE], 0.002 [0.001]; *P* = .005; and alternative IL-6 signaling weighted median estimate [SE], 0.002 [0.001]; *P* = .047) (Table 3 and eTable 12 and eTable 13 in the Supplement). There was also some evidence in IVW but not weighted median MR analysis for associations of (indirect) IL-6 signaling with sleep problems, but not with insomnia, suggesting potential association specificity to hypersomnia (Table 3).

Findings for BMI

In IVW MR analyses, the instrument used for BMI indicated that higher BMI was associated with anhedonia, tiredness, changes in appetite, and feelings of inadequacy (estimate [SE], 0.046 [0.012]; FDR *P* = .001 for anhedonia; estimate [SE], 0.049 [0.018]; FDR *P* = .02 for tiredness; estimate [SE], 0.121 [0.013]; FDR *P* < .001 for changes in appetite; and estimate [SE], 0.028 [0.011]; FDR *P* = .02 for feelings of inadequacy) (Figure 2 and eTable 11 and eFigure 3 in the Supplement). Except for feelings of inadequacy, these associations persisted in weighted median MR analyses (estimate [SE], 0.042 [0.016]; *P* = .007 for anhedonia; estimate [SE], 0.057 [0.023]; *P* = .01 for tiredness; estimate [SE], 0.141 [0.017]; *P* < .001 for changes in appetite; and estimate [SE], 0.031

[0.016]; P = .06 for feelings of inadequacy) (eTable 12 in the Supplement).

Assessment of Horizontal Pleiotropy

Assessing if SNV-outcome associations are mediated via the exposure and not via other mechanisms (ie, horizontal pleiotropy) is a key prerequisite for validity of causal inference from MR analysis. As detailed in the eResults in the Supplement, we assessed horizontal pleiotropy by measuring between-SNV heterogeneity (eTable 14 and eTable 15 in the Supplement), and we performed sensitivity analyses that are more robust to pleiotropy, including gene-restricted MR, MR-Egger regression, and MR analyses excluding outlying pleiotropic SNVs (eFigure 4 and eTables 16-20 in the Supplement). The association of IL-6 signaling with suicidality was robust across sensitivity analyses (main IL-6 signaling Cochran Q = 5.77; P = .33; gene-restricted IVW estimate [SE], 0.027 [0.011]; P = .01). The associations of higher BMI with anhedonia, tiredness, changes in appetite, and feelings of inadequacy were directionally consistent in all sensitivity analyses (MR-Egger slope [SE], 0.028 [0.036]; P = .44 for anhedonia; MR-Egger slope [SE], 0.043 [0.056]; P = .45 for tiredness; MR-Egger slope [SE], 0.183 [0.038]; P < .001 for changes in appetite; and MR-Egger slope [SE], 0.050 [0.033]; P = .14) for feelings of inadequacy.

Discussion

We tested SNV-based genetic correlation and potential MR association between proinflammatory activity and individual depressive symptoms. Using LDSC regression, we showed consistent genetic correlations between CRP levels, a sensitive index of inflammatory activity, and depressive symptoms as assessed with the PHQ-9. Genetic correlations between CRP levels and each specific depressive symptom were small and similar in size (genetic correlation range, 0.152-0.362). Mendelian randomization analyses for specific depressive symptoms showed consistent evidence for associations between higher IL-6 activity and suicidality. Findings of increased CRP levels and IL-6 overactivity with other PHQ-9 symptoms were inconsistent, but there were some indications that IL-6 signaling could be associated with hypersomnia, which requires replication in future work. Regarding metabolic dysregulation and depressive symptoms, we found consistent MR associations of higher BMI with anhedonia, tiredness, changes in appetite, and feelings of inadequacy. However, our MR analyses did not replicate prior research showing MR associations of CRP levels or IL-6 signaling with MD[31] (eDiscussion in the Supplement).

Inflammation and Suicidality

Suicidality and suicidal behavior have a multifactorial origin, and identification of causal markers is critical to advance prevention and treatment efforts.[64,65] Increased levels of inflammatory markers, and IL-6 in particular, have been found to be associated with suicidality or suicidal behavior, and patients with chronic inflammatory illnesses, such as inflammatory bowel disease, exhibit increased suicide rates.[66-69] We provide evidence for an association between higher IL-6 signaling and suicidality. Findings of this association were consistent across LDSC regression and MR analyses using different genetic proxies for IL-6 signaling.

An association between IL-6 signaling and suicidality may have important clinical implications. First, suicidality may become a useful symptom (characteristic of inflammatory activity beyond CRP levels) for stratification efforts in RCTs of immunotherapy in depression. Second, it may be informative to evaluate the symptom-specific effectiveness of immunotherapies for depression and for treating suicidality in particular. Raison and colleagues[15] demonstrated the symptom-specific effectiveness of the tumor necrosis factor α inhibitor infliximab for suicidality (among 4 other symptoms) in patients with MD with high CRP levels before treatment. Data from available RCTs of immunotherapies for chronic inflammatory illnesses and from RCTs of anti–IL-6 and anti–IL-6R drugs in MD[17] may be valuable to further examine symptom-specific immunotherapy outcomes. Therefore, our findings emphasize the need for considering suicidality in immunopsychiatry research and highlight the clinical potential of immunotherapies, and specifically IL-6R blockade, for treatment of suicidality.

Inflammation, Metabolic Dysregulation, and Depressive Symptoms

Apart from suicidality and preliminary indications for hypersomnia, results for inflammation and other PHQ-9 depressive symptoms were divergent between LDSC regression, in which robust genetic correlations between CRP levels and depressive symptoms were found, and MR analyses, in which inconsistent associations that did not replicate across instruments or statistical models were found (Table 3 and eTables 11-17 in the Supplement). This dissociation of genetic correlation and MR results for other PHQ-9 depressive symptoms may offer important new insights into the interrelationship between low-grade inflammation, metabolic dysregulation, and depression.

Mendelian randomization associations of higher BMI, but not of increased inflammatory markers, with anhedonia, tiredness, changes in appetite, and feelings of inadequacy suggest that metabolic dysregulation may underlie the coheritability of inflammatory activity with these symptoms. In the context of previous results,[4,70-72] it is likely that associations with changes in appetite are specific to increased appetite or hyperphagia. Taken together, these results suggest immune and metabolic factors may constitute separate, symptom-specific risk factors in depression.

Because depressive symptoms themselves could promote proinflammatory lifestyle choices, such as an unhealthy diet and reduced physical activity,[73,74] reverse causal inference needs to be assessed as an alternative or additional explanation. Future studies need to replicate our results and should further investigate pleiotropic or residual confounding factors that could explain genetic correlations between CRP levels and BMI with depressed mood, concentration problems, and psychomotor changes. In these studies, symptoms should ideally be assessed without composite items and based on multiple symptom indicators.

Strengths and Limitations

We report genetic analyses of inflammatory activity, metabolic dysregulation, and depressive symptoms based on large GWAS data sources. Our sample size maximizes power for genetic analyses.

This study also has some limitations. First is a lack of granular information on some depressive symptoms assessed by the PHQ-9, which does not differentiate between diametrically opposite symptoms. Although inclusion of insomnia summary data helped in providing some preliminary suggestions on associations between IL-6 and hypersomnia, more detailed investigations disentangling composite symptoms are needed.

Second, depressive symptoms in the general population and in patients with MD are likely to exist on a continuum, but they could also be different with regard to their origin and implications, especially because depressive symptoms are common and may arise in the general population owing to a variety of reasons other than depression.[75] In future work, MR analysis of symptoms in patients with MD is required to assess the relevance of our findings for depressive symptoms occurring in the context of MD.

Third, inferences on causality should ideally rely on multiple types of studies because MR analyses rely on 3 key assumptions that are not always met or completely testable.[57,76] Ohlsson and Kendler[77] have advocated triangulation of causality using different study designs, such as MR and RCTs, which we also support.

Fourth, our IL-6 signaling instrument was weighted on downstream associations with CRP levels, and it is debatable if this approach captures an independent association of IL-6 signaling. However, the fact that we replicated our results using an sIL-6R–based instrument supports this interpretation.

Conclusions

This genetic correlation and 2-sample MR study reports a detailed investigation of inflammatory activity, metabolic dysregulation, and specific depressive symptoms using LDSC regression and 2-sample MR analyses of large GWAS data. The findings suggest small but robust genetic correlations of BMI and CRP levels with depressive symptoms, and MR associations show that higher BMI could be a causal risk factor for anhedonia, tiredness, changes in appetite, and feelings of inadequacy. Regarding proinflammatory processes, IL-6 signaling may be potentially causally associated with suicidality. This hypothesis is clinically relevant because symptom expression of suicidality could help identify patients who will respond to immunotherapy. The findings also suggest that pharmacological approaches targeting IL-6 signaling may be valuable for treatment of suicidality, which requires further research.

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Correction: This article was corrected on December 2, 2020, to fix the CRP level axis in Figure 2.

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3. Polygenic risk for immuno-metabolic markers and specific depressive symptoms

3.1 Summary

To pinpoint etiological pathways between inflammation and depression, the objective of Study 2 was to evaluate associations between polygenic risk for higher immuno-metabolic markers and specific depressive symptoms and to test if these associations are direct or indirect. To meet these objectives, we used data from three large samples, the general population-based UK Biobank study, the MDD outpatient Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study, and the MDD inpatient Munich Antidepressant Response Signature (MARS) study. Samples were restricted to individuals from European descent and with available data on seven depressive symptoms (i.e., depressed mood, anhedonia, sleep problems, fatigue, changes in appetite, psychomotor changes, & suicidality) and genotype information. This resulted in final sample sizes of 110,010 individuals from UK Biobank, 1,143 outpatients from STAR*D, and 1,058 inpatients from MARS samples. Using discovery GWAS data for five immuno-metabolic markers (C-reactive protein [CRP], interleukin [IL]-6, IL-10, tumour necrosis factor [TNF]-α, BMI), polygenic risk scores (PRSs) for participants were computed using the Bayesian regression and continuous shrinkage priors (PRS-CS) approach. Network analysis was applied on symptom and PRS data using the fused graphical least absolute shrinkage and selection operator (FGL) algorithm, which jointly estimates networks across samples, and (as secondary analysis) unregularized model search estimation. Three consistency criteria were defined to evaluate findings in terms of consistency across samples, statistical bootstraps, and estimation algorithms.

Results from these analyses showed a unique association of the CRP PRS with changes in appetite that met all three consistency criteria. Meeting two consistency criteria, we also observed unique associations between higher polygenic risk for CRP with greater fatigue and reduced anhedonia; between higher polygenic risk for TNF-α with greater fatigue; and between higher polygenic risk for BMI with greater changes in appetite and anhedonia. These results align with previous literature suggesting that immuno-metabolic alterations are primarily associated with neurovegetative symptoms of depression such as changes in appetite and fatigue among others. They also extend findings from previous studies by demonstrating these associations replicate for PRSs for immuno-metabolic markers (e.g., as compared to serum cytokine measurements) and that associations are direct and unlikely to be fully mediated by other depressive symptoms. Taken together, these findings could inform future clinical trials of anti-inflammatory drugs or lifestyle

interventions for depression that could benefit from selective recruitment of patients with an atypical/ neurovegetative symptom profile.

3.2 Contributions and reference

The study "Polygenic risk for immuno-metabolic markers and specific depressive symptoms: A multi-sample network analysis study" was published in Brain, Behavior and Immunity in March, 2021. NK, EBB, GMK, and JA were responsible for concept and design; NK, DC, NR, SM, VS, LT, JS, EBB, GMK, and JA for acquisition, analysis, or interpretation of data; NK, JS, and JA for statistical analysis; NK for drafting of manuscript; CHARGE inflammation working group for administrative, technical, or material support; NK, DC, NR, SM, VS, LT, JS, SL, EBB, GMK, and JA for critical revision of content; and DC, EBB, GMK, and JA for supervision.

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Polygenic risk for immuno-metabolic markers and specific depressive symptoms: A multi-sample network analysis study

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ABSTRACT

Background: About every fourth patient with major depressive disorder (MDD) shows evidence of systemic inflammation. Previous studies have shown inflammation-depression associations of multiple serum inflammatory markers and multiple specific depressive symptoms. It remains unclear, however, if these associations extend to genetic/lifetime predisposition to higher inflammatory marker levels and what role metabolic factors such as Body Mass Index (BMI) play. It is also unclear whether inflammation-symptom associations reflect direct or indirect associations, which can be disentangled using network analysis.

Methods: This study examined associations of polygenic risk scores (PRSs) for immuno-metabolic markers (C-reactive protein [CRP], interleukin [IL]-6, IL-10, tumour necrosis factor [TNF]-α, BMI) with seven depressive symptoms in one general population sample, the UK Biobank study (n = 110,010), and two patient samples, the Munich Antidepressant Response Signature (MARS, n = 1058) and Sequenced Treatment Alternatives to Relieve Depression (STAR*D, n = 1143) studies. Network analysis was applied jointly for these samples using fused graphical least absolute shrinkage and selection operator (FGL) estimation as primary analysis and, individually, using unregularized model search estimation. Stability of results was assessed using bootstrapping and three consistency criteria were defined to appraise robustness and replicability of results across estimation methods, network bootstrapping, and samples.

Results: Network analysis results displayed to-be-expected PRS-PRS and symptom-symptom associations (termed edges), respectively, that were mostly positive. Using FGL estimation, results further suggested 28, 29, and six PRS-symptom edges in MARS, STAR*D, and UK Biobank samples, respectively. Unregularized model search estimation suggested three PRS-symptom edges in the UK Biobank sample. Applying our consistency criteria to these associations indicated that only the association of higher CRP PRS with greater changes in appetite fulfilled all three criteria. Four additional associations fulfilled at least two consistency criteria; specifically, higher CRP PRS was associated with greater fatigue and reduced anhedonia, higher TNF-α PRS was associated with greater fatigue, and higher BMI PRS with greater changes in appetite and anhedonia. Associations of the BMI PRS with anhedonia, however, showed an inconsistent valence across estimation methods.

Conclusions: Genetic predisposition to higher systemic inflammatory markers are primarily associated with somatic/neurovegetative symptoms of depression such as changes in appetite and fatigue, consistent with previous studies based on circulating levels of inflammatory markers. We extend these findings by providing evidence that associations are direct (using network analysis) and extend to genetic predisposition to immuno-metabolic

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markers (using PRSs). Our findings can inform selection of patients with inflammation-related symptoms into clinical trials of immune-modulating drugs for MDD.

1. Introduction

Recent findings suggest that every fourth patient with Major Depressive Disorder (MDD) shows evidence of systemic, low-grade inflammation as indicated by elevated (>3mg/L) C-reactive protein (CRP) concentrations (Osimo et al., 2019). This association has been supported by cross-sectional case-control studies synthesised in multiple meta-analyses (Dowlati et al., 2010; Goldsmith et al., 2016; Haapakoski et al., 2015; Howren et al., 2009; Köhler et al., 2017) as well as longitudinal studies (Khandaker et al., 2014; Lamers et al., 2020; Mac Giollabhui et al., 2020). Clinically, patients with evidence of inflammation do not respond as well to standard monoaminergic and psychotherapeutic treatments (Liu et al., 2020; Lopresti, 2017). These patients may, however, benefit from alternative treatment with immune-modulating drugs (Kappelmann et al., 2018; Köhler-Forsberg et al., 2019; Wittenberg et al., 2020). To prioritise drug and patient selection for clinical trials, it is crucial to further understand immunological and clinical complexity of inflammation-symptom associations, which may allow shortlisting of promising immunotherapeutic drug targets and could highlight patients with a profile of inflammation-related depression.

Regarding immunological complexity, studies have reported various associations of serum inflammatory proteins with depression, including among others CRP, interleukin (IL)-6, IL-10, and tumour necrosis factor (TNF)-α (Goldsmith et al., 2016; Haapakoski et al., 2015; Köhler et al., 2017). Evidence from in-depth immunophenotyping further suggests that there may be distinct subgroups of inflammation-related depression as shown by immune cell count clustering and transcriptome analyses (Cattaneo et al., 2020; Lynall et al., 2020). These studies suggest that elevated serum levels of inflammatory markers are associated with depression, but associations of depression with genetic/lifetime predisposition to higher inflammatory markers has been studied less frequently and primarily for CRP (Badini et al., 2020; Kappelmann et al., 2021; Milaneschi et al., 2017b, 2016). Elevated serum levels of inflammatory markers also conflate tonic and phasic levels of inflammatory markers while genetic/lifetime predisposition to inflammatory markers specifically maps their tonic levels. This differentiation could be relevant as highlighted by research into tonic versus phasic dopamine levels (see Bilder et al., 2004), whereby tonic levels regulate the amplitude of the phasic response, which has unique consequences for downstream signalling. Lastly, inflammatory markers such as CRP are influenced by metabolic factors (Timpson et al., 2011), which may causally underlie some inflammation-symptom associations (Kappelmann et al., 2021), so a combined investigation of immuno-metabolic factors is needed to disentangle their etiological roles.

Regarding clinical complexity, most prior research has restricted its investigation of the inflammation-depression association to complexity on one side, that is focusing on multiple immune markers (e.g., cell counts/ serum cytokine levels) while studying a composite depression phenotype (Goldsmith et al., 2016; Haapakoski et al., 2015; Köhler et al., 2017) or focusing on multiple depressive symptoms or symptom groups in the context of a single inflammatory marker (mostly CRP) (Badini et al., 2020; Jokela et al., 2016; Köhler-Forsberg et al., 2017; Lamers et al., 2020, 2019; White et al., 2017). Among studies focusing on individual symptoms, results have highlighted associations of inflammatory markers with specific depressive symptoms of fatigue, changes in appetite, anhedonia, and suicidality (Badini et al., 2020; Chu et al., 2019; Jokela et al., 2016; Kappelmann et al., 2021; Köhler-Forsberg et al., 2017; Lamers et al., 2020, 2018; Milaneschi et al., 2017a; Simmons et al., 2018; White et al., 2017). However, most of these studies have considered associations of inflammatory markers with each depressive symptom in isolation (Chu et al., 2019; Jokela et al., 2016; Kappelmann et al., 2021; Köhler-Forsberg et al., 2017; Lamers et al., 2018; White et al., 2017). Although these prior approaches have led to important findings, they cannot address potential causal interactions between symptoms, thus conflate evidence for indirect and direct associations. For example, analyses of isolated symptoms could hypothetically provide evidence for associations of CRP with both fatigue and sleep problems even if CRP was only indirectly associated with fatigue *via* its effect on sleep problems. A network-based approach provides one means of disentangling such direct from indirect inflammation-symptom associations.

Network theory and related analysis techniques have recently been put forward to accommodate the symptomatic complexity of mental disorders (Borsboom, 2017). Network theory proposes putative causal interactions between symptoms (e.g., fatigue *causing* concentration problems *causing* low mood), which could result in self-reinforcing vicious symptom cycles triggering and maintaining mental disorders. Such associations have been investigated in an increasing amount of studies on psychological symptom networks (Contreras et al., 2019; Robinaugh et al., 2020). To accommodate etiological factors beyond symptoms, however, recent work has proposed an expansion of symptom networks to so-called 'multi-plane' networks, for instance also including genetic, metabolic, immunological, or environmental variables (Guloksuz et al., 2017). To our knowledge, so far, two studies have evaluated such multi-plane networks in the context of inflammation and depression by jointly analysing serum CRP (plus IL-6 & TNF-α in the study of Fried et al., 2019), BMI, and potential covariates with individual depressive symptoms (Fried et al., 2019; Moriarity et al., 2020a). The most consistently replicated findings between these two studies suggested unique associations of CRP with fatigue and changes in appetite. A third study has recently also provided evidence that the symptom structure itself was a function of CRP levels; that is, interconnections between symptoms were moderated by CRP (Moriarity et al., 2020b). All of these previous studies were based on serum markers for inflammatory proteins, however, reflective of acutely elevated inflammatory activity. Therefore, it remains unclear if inflammation-symptom associations generalise to genetic/lifetime predisposition to higher immuno-metabolic marker levels.

In the present study, we explored associations of polygenic risk scores (PRSs) for four major pro- and anti-inflammatory markers (i.e., CRP, IL-6, IL-10, & TNF-α) and Body Mass Index (BMI), as a metabolic marker, with individual depressive symptoms using a multi-sample, multi-plane network analysis approach. We evaluated associations in three large samples including the inpatient Munich Antidepressant Response Signature (MARS) study (n = 1,058), the outpatient Sequenced Treatment Alternatives to Relieve Depression (STAR*D) study (n = 1,143), and the general population UK Biobank cohort (n = 110,010) (Hennings et al., 2009; Rush et al., 2004; Sudlow et al., 2015). This investigation aimed to contribute to the study of inflammation and depression by simultaneously addressing (i) combined immunological and symptom complexity (using network analysis), (ii) unclarity regarding the influence of genetic/lifetime predisposition to higher immuno-metabolic marker levels on depression (defining immuno-metabolic markers using PRSs), and (iii) issues of replicability and generalisability (testing associations in one large general population and two clinical samples).

2. Methods

An overview of the study design and analytic procedure is presented in Fig. 1.

2.1. Study samples

The Munich Antidepressant Response Signature (MARS) study was a naturalistic, observational study of inpatients with major depressive disorder (MDD) or bipolar disorder conducted between 2000 and 2015 in three Southern German hospitals (Hennings et al., 2009). Based on an original sample of 1,411 patients, the present study included 1,058 patients of European descent with an ICD diagnosis of MDD (F32 and F33 codes) and genetic and depressive symptom data.

The STAR*D trial (identifier: NCT00021528) was a multisite, multistep, randomised controlled trial (RCT), conducted from 2000 to 2004, evaluating different treatment options and sequences for outpatients suffering from DSM-IV MDD without psychotic features (Rush et al., 2004). Based on an original sample of 1,953 patients who took part in the STAR*D genetics study, the present study included 1,143 individuals of European descent with genetic and depressive symptom data.

The UK Biobank is a general population cohort including >500,000

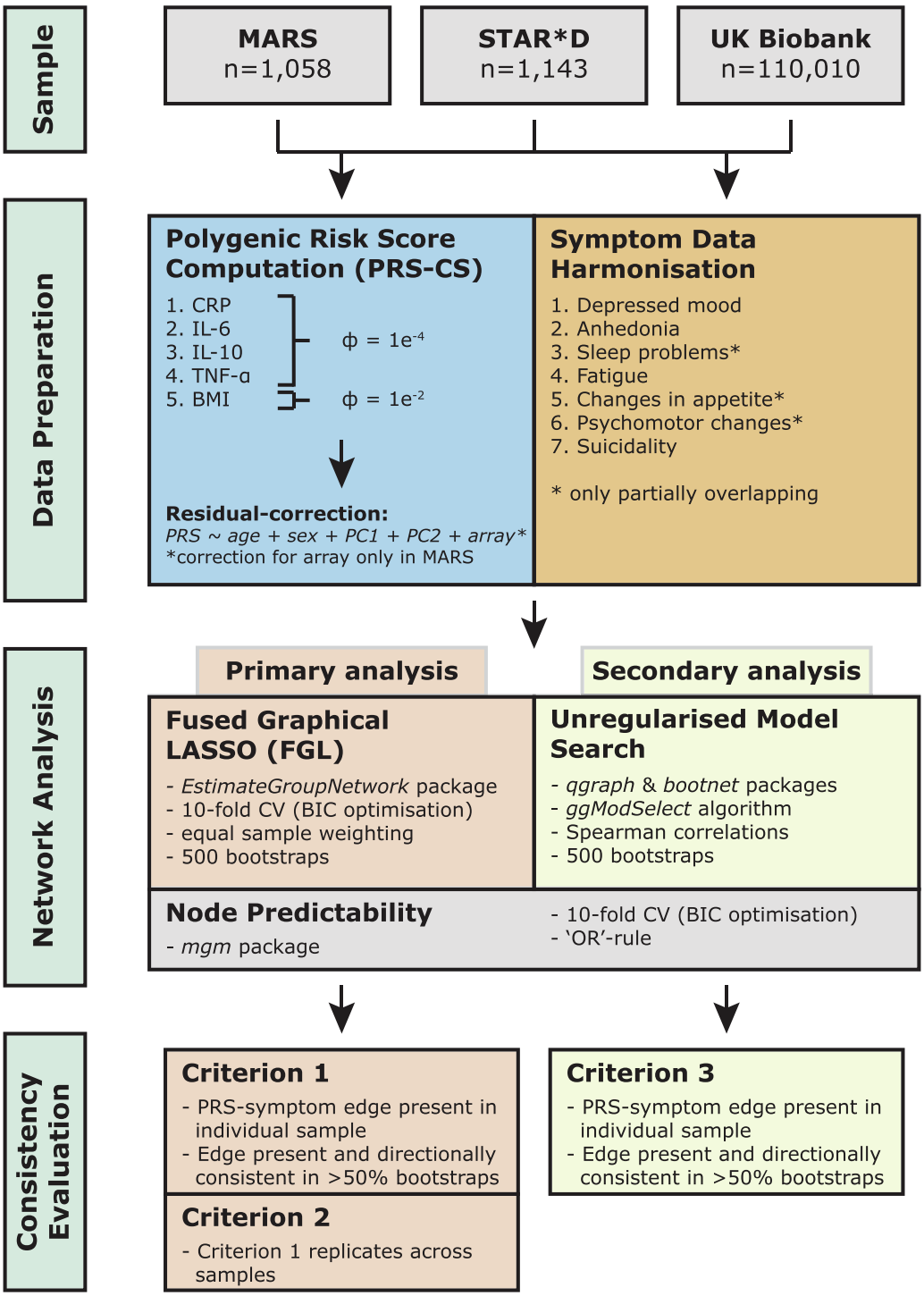

Fig. 1. Study design and analysis pipeline. BIC = Bayesian information criterion; CV = cross-validation; PC = principal component (or multi-dimensional scaling component used for MARS & STAR*D); ϕ = PRS-CS tuning parameter.

individuals, recruited from 2006 to 2010, with genotyping and in-depth phenotyping information (Bycroft et al., 2018). About 157,000 individuals from the initial sample took part in a follow-up mental health survey (Davis et al., 2020) and we included a subset of 110,010 individuals that were of European descent and had available genetic and depressive symptom data.

2.2. Ethics approval and informed consent

MARS received local ethics approval from Ludwig Maximilians University Munich (Hennings et al., 2009). STAR*D received ethics approval from 14 participating institutional review boards, a National Coordinating Center, a Data Coordinating Center, and the Data Safety and Monitoring Board at the National Institute of Mental Health (Rush et al., 2006, 2004). The UK Biobank study received ethics approval from North West Centre for Research Ethics Committee and Human Tissue Authority research tissue bank (Bycroft et al., 2018); this project was approved under project no. 26999. All three studies collected informed consent from participants prior to study participation.

2.3. Depressive symptom assessment

Depressive symptoms were assessed differently across the three samples. MARS and STAR*D studies used the observer-rated Hamilton Rating Scale for Depression (HAM-D) (Hamilton, 1986) while the UK Biobank study used the self-report Patient Heath Questionnaire (PHQ)-9 (Löwe et al., 2004). From these questionnaires, we selected seven depressive symptoms for joint analyses across samples. These symptoms included completely overlapping symptoms of depressed mood, anhedonia, fatigue, and suicidality, but also partially overlapping symptoms of sleep problems, changes in appetite, and psychomotor changes. Supplementary Table 1 provides an item-level overview of depressive symptoms and Supplementary Table 2 displays symptom coding, where this differed from original Likert scale ratings.

Regarding partially overlapping symptoms of sleep problems, changes in appetite, and psychomotor changes, the PHQ-9 only assesses information on conflated symptoms (e.g., insomnia and hypersomnia are conflated to sleep problems) while the HAM-D incorporates disaggregated symptoms. To harmonise these symptom data for retention in network analyses, we conflated HAM-D symptoms of psychomotor retardation and agitation to "psychomotor changes". For sleep problems and changes in appetite (available in the PHQ-9), only insomnia and loss of appetite are available in the HAM-D, so we included both conflated and unidirectional symptoms in network analyses as previous studies have specifically highlighted associations of inflammation with these symptoms (Jokela et al., 2016; Milaneschi et al., 2017b). We reasoned that comparative appraisal of associations, for example with changes in appetite and loss of appetite, could give further indications on potential specificity of associations to symptom directions, as observed in previous reports (Kappelmann et al., 2021; Milaneschi et al., 2021b, 2021a).

We also note that we have not included items of "guilt or self-blame" from the respective studies in our analyses as we considered the item content of HAM-D and PHQ-9 too distinct. Specifically, the HAM-D conflates feelings of guilt with delusions of guilt and death, thus moving towards psychotic symptomatology. Contrary to this, the PHQ-9 also includes "feelings of inadequacy" about oneself, which are not covered by the HAM-D item.

2.4. Genotyping, quality control and imputation

We provide detailed information on genotyping, quality control and imputation procedures in the Supplementary Methods. Briefly, genotyping in the MARS study was conducted using three genotyping arrays across the recruitment period (see Supplementary Fig. 1), the Illumina 610 k (n = 548), Illumina OmniExpress (n = 284) and Illumina GSA (n = 226) arrays. In STAR*D, genotyping was conducted using the Affymetrix Human Mapping 500 K Array Set (n = 979) and the Affymetrix Genome-Wide Human SNP Array 5.0 (n = 969) that displayed a concordance of > 99%; described in detail by Garriock et al. (2010). In the UK Biobank study, samples were genotyped on the UK BiLEVE Axiom Array or the Affymetrix UK Biobank Axiom Array (Bycroft et al., 2018). Following imputation in all samples, single nucleotide polymorphisms (SNPs) with info-metric > 0.6, minor allele frequency (MAF) > 1%, genotyping missingness < 2%, and no deviation from Hardy-Weinberg Equilibrium (MARS & STAR*D: $P > 1e^{-5}$; UK Biobank: $P > 1e^{-7}$) were retained.

2.5. Polygenic risk scores

2.5.1. Immuno-metabolic marker selection and GWAS data sources

PRSs for CRP, IL-6, IL-10, TNF-α, and BMI were computed based on available summary statistics from genome-wide association studies (GWAS; Ahola-Olli et al., 2017; Ligthart et al., 2018; Locke et al., 2015). These inflammatory markers were selected, because (i) they showed robust differences in case-control studies; (ii) CRP, IL-6, and TNF-α have been the most frequently investigated inflammatory markers overall in the context of depression; and (iii) IL-10 was the most frequently studied anti-inflammatory cytokine, so could be informative on direction of associations between depressive symptoms and innate immune activity (Köhler et al., 2017; Osimo et al., 2019). BMI was selected as the most frequently investigated metabolic marker.

GWAS data for CRP were obtained from a large GWAS of 88 studies including 204,402 individuals of European descent (Ligthart et al., 2018). GWAS data for IL-6, IL-10, and TNF-α were obtained from a GWAS of 8,293 Finns (Ahola-Olli et al., 2017); of note, Finns have Siberian ancestry (Lamnidis et al., 2018), which leads to a divergence from European ancestry of our analytic samples. GWAS data for BMI were obtained from the Genetic Investigation of Anthropometric Traits (GIANT) consortium that included up to 322,154 individuals of European descent (Locke et al., 2015).

2.5.2. PRS computation

PRSs can be computed by summing the GWAS association estimates of risk alleles for each individual. Classically, this summation is done using an approach termed "clumping and thresholding" (C + T), which first reduces summary statistics to independent SNPs and then applies one or multiple thresholds (usually based on P-values) to restrict summation to SNPs with high evidence for associations with phenotypes (Choi et al., 2020). As the optimal threshold for the C + T approach is unknown and should ideally be estimated in a separate dataset with available phenotype data, we computed PRSs using the Bayesian regression and continuous shrinkage priors (PRS-CS) approach, which has been shown to perform similar to or outperform other PRS computation approaches such as C + T (Ge et al., 2019; Ni et al., 2020).

PRS-CS takes a linkage disequilibrium (LD) reference panel into account (we used European ancestry data from 1000 Genomes Project phase 3 samples) to update SNP effect sizes in a blocked fashion, thus providing accurate LD adjustment. We pre-specified the global shrinkage parameter ϕ using suggested defaults for less polygenic ($\phi = 1e^{-4}$) and more polygenic ($\phi = 1e^{-2}$) phenotypes as $\phi = 1e^{-4}$ for CRP, IL-6, IL-10, and TNF-α, and as $\phi = 1e^{-2}$ for BMI; see details in Supplementary Methods. Following PRS computation in individual samples, polygenic scores were corrected for age, sex, and the first two genotyping principal or multidimensional scaling (MDS) components using linear regression; two genotyping principal or MDS components were selected as visual inspection of component inter-correlations did not suggest evidence for population stratification. Genotyping MDS components were computed based on raw Hamming-distances in MARS and STAR*D, and using principal component analysis on high-quality, unrelated individuals in the UK Biobank sample (Bycroft et al., 2018). PRSs in MARS were additionally corrected for the genotyping array. Following computation, higher PRSs reflect higher genetic predisposition to respective immuno-

metabolic phenotype levels.

2.5.3. PRS evaluation

In Supplementary Table 3, we provide the number of SNPs included in PRS computation in each sample, which was approximately around one million SNPs for each phenotype-sample combination. The proportion of SNP overlap between samples (for the same phenotype) was > 0.89 suggesting that mostly overlapping SNPs contributed to PRSs (Supplementary Table 4). Taking these overlapping SNP sets, correlations between the posterior SNP effect sizes between samples were large for CRP (Pearson's *r* range: 0.69–0.76) and BMI (Pearson's *r* range: 0.79–0.80) and relatively smaller for IL-6, IL-10, and TNF-α (Pearson's *r* range: 0.41–0.46; see Supplementary Table 5). This suggests polygenic risk was quantified more similarly across samples for CRP and BMI as compared to IL-6, IL-10, and TNF-α.

We quantified the impact that pre-specification of the hyperparameter ϕ had on resulting PRSs, which was likely small (Supplementary Table 6). Specifically, PRSs with pre-specified ϕ exhibited large correlations with PRSs based on automatic learning of ϕ from GWAS summary data (termed PRS-CS-auto in the literature; Pearson's *r* range: 0.82–0.98). Furthermore, moderate-to-large correlations remained to PRSs based on extreme grid search boundary values of ϕ (Pearson's *r* range: 0.47–0.93).

Since MARS utilised three different genotyping arrays, we verified that our approach of combining data from these arrays into one sample was justified before proceeding with the main analysis (see Supplementary Methods and Supplementary Figs. 2 and 3).

2.6. Network analysis

2.6.1. Estimation

Network analysis was conducted using *R* software (version 4.0.3; R Core Team, 2017). In network analysis, unique associations between variables reflect partial correlations and are termed 'edges'. Variables in the network are referred to as 'nodes'.

Network models can be broadly categorised into regularized and unregularized models, that have distinct advantages and disadvantages. Regularised models apply penalties that shrink edges towards zero. This has the advantage that it results in sparser and more parsimonious network models as small edges can be set exactly to zero. Contrary to this, non-regularized models do not apply such a penalty- while it is still possible to control the false positive rate- and recent studies have suggested that unregularized models perform better in estimating psychological symptom networks and multi-plane immunopsychiatric networks than regularized network models (Moriarity et al., 2020a; Williams et al., 2019). A disadvantage of unregularized models, however, is that they are currently only suitable for network estimation of individual samples/datasets. Contrary to this, regularised models have recently been adapted for application in multi-sample contexts using so-called fused graphical LASSO (FGL) estimation. FGL estimation allows synthesising data across multiple samples, which increases statistical power.

Based on these respective advantages and disadvantages, we have decided to use a regularized network model as primary analysis, which maximises statistical power due to the multi-sample design of our study. As unregularized models are preferable for estimation of individual samples and may be better suited to retrieve multi-plane edges, however, we also apply unregularized network estimation as secondary analysis.

In primary analyses, networks were estimated using FGL estimation as implemented in the *EstimateGroupNetwork* package (version 0.2.2; Costantini et al., 2020, 2019; Danaher et al., 2014). FGL estimation relies on the two tuning parameters λ_1, which penalizes network density, and λ_2, which penalizes edge differences across samples. Values for these tuning parameters were selected using 10-fold cross-validation to optimise the Bayesian Information Criterion (BIC). As recommended, we set weights for the importance of each sample as 'equal' to ascertain that a single sample would not dominate estimation (Danaher et al., 2014).

As secondary analysis, we estimated unregularized networks for each sample individually using the gaussian graphical stepwise model selection ("ggModSelect") algorithm implemented in the *qgraph* package (version 1.6.5; Epskamp et al., 2012) based on Spearman correlations and starting from an empty model. Throughout results, we refer to this estimation strategy as "unregularized model search" or "model search" for simplification.

2.6.2. Node predictability

We also estimated node predictability, which describes the amount of variance in a node that is explained by all other nodes in the network, so can be interpreted akin to R^2 (Haslbeck and Fried, 2017). Node predictability cannot be inferred from FGL or model search networks as it requires a node-wise estimation approach. Therefore, we used a mixed graphical model as a third estimation strategy as implemented in the *mgm* package (version 1.2–10), selecting tuning parameter λ based on BIC optimisation in 10-fold cross-validation (Haslbeck and Waldorp, 2020). Of note, this model was only used to infer node predictability, which provides additional information on network density and sample comparability. However, we do not report any individual edge estimates based on this model as FGL estimation and unregularized model search are better suited for our study aims.

2.6.3. Visualisation

Networks were visualised with the *qgraph* package using an average layout estimated with the Fruchterman-Reingold algorithm for the FGL networks. This algorithm places nodes close to each other that are connected by large edges (Epskamp et al., 2012). While this simplifies network appraisal, it is important to note that nodes and edges should not be interpreted based on their relative position within the network, which can be unstable.

2.6.4. Stability

To evaluate stability of estimated networks, we assessed accuracy of edge estimates using bootstrapping strategies. Specifically, for FGL networks 500 bootstrapped samples with replacement were drawn, and FGL networks re-estimated, using the implementation in the *EstimateGroupNetwork* package (Costantini et al., 2020). For unregularized model search estimation, the same procedure was applied using non-parametric bootstrapping procedures implemented in the *bootnet* package (version 1.4.3; Epskamp et al., 2018).

2.6.5. Interpretation

We interpreted estimated networks based on the presence, stability, and replicability of edges as defined using three consistency criteria. First, we tested if edges were nonzero in FGL networks as well as nonzero and directionally consistent in $> 50\%$ of bootstrapped analyses (consistency criterion 1) akin to a previous PRS-symptom network study in psychosis by Isvoranu and colleagues (2020). Second, we tested if edges between PRSs and symptoms replicated (according to criterion 1) across FGL networks of the three samples (consistency criterion 2). Third, we tested if edges were present in secondary analyses using unregularized model search estimation, again confirmed in $> 50\%$ of bootstrapped estimations exhibiting directionally consistent estimates (consistency criterion 3).

2.7. Availability of data and materials

Data from original studies is not openly available, but can be requested; see details in Supplementary Table 7. GWAS summary data for IL-6, IL-10, and TNF-α is openly available from the original publication by Ahola-Olli and colleagues (2017), for BMI from the GIANT consortium, and can be requested for CRP from the CHARGE inflammation working group. We provide analysis scripts and estimated

network matrices (including bootstrapped network matrices) on the Open Science Platform (OSF) under https://osf.io/q4vw9/.

3. Results

Baseline characteristics of study populations are displayed in Table 1.

3.1. Network analysis

We conducted network analyses of five immuno-metabolic PRSs (CRP, IL-6, IL-10, TNF-α, & BMI) and seven depressive symptoms using two estimation techniques (FGL & unregularized model search estimation) in three samples (MARS, STAR*D & UK Biobank). Bootstrap analyses were conducted to assess stability of networks and node predictability estimated using a mixed graphical model. We defined three consistency criteria to assess robustness and replicability of our results across estimation techniques, bootstrapping, and samples. Focus of this network investigation were unique associations (termed edges in network analysis) between PRSs and symptoms, which are summarised in Table 2.

Table 1
Baseline characteristics of MARS, STAR*D, and UK Biobank samples.

	MARS	STAR*D	UK Biobank
N	1,058	1,143	110,010
Sex			
Women, N (%)	563 (53.2%)	676 (59.1%)	61,212 (55.6%)
Men, N (%)	495 (46.8%)	467 (40.9%)	48,798 (44.4%)
Age in years			
Mean (SD)	47.8 (14.4)	43.2 (13.6)	56.2 (7.7)
Range	18–87	18–75	39–72
Study location	Germany	United States	United Kingdom
Study population	MDD inpatients	MDD outpatients	General population
CRP PRS			
Mean (SD)	−0.01 (1.00)	0.00 (1.00)	−0.01 (1.00)
Range	−3.14–3.15	−3.21–2.94	−4.58–4.21
IL-6 PRS			
Mean (SD)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Range	−3.33–3.34	−3.41–3.94	−4.07–3.92
IL-10 PRS			
Mean (SD)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Range	−3.11–3.24	−3.28–3.13	−4.35–4.60
TNF-a PRS			
Mean (SD)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
Range	−3.61–3.67	−3.35–3.52	−4.17–4.51
BMI PRS			
Mean (SD)	−0.02 (1.01)	0.00 (1.00)	−0.04 (1.00)
Range	−3.02–4.06	−3.04–3.20	−4.53–3.96
PHQ-9 sum-score			
Mean (SD)	–	–	2.7 (3.6)
Range	–	–	0–27
Missing, N (%)	–	–	351 (0.3)
HAM-D sum-score			
Mean (SD)	23.8 (5.9)	22.4 (4.9)	–
Range	5–42	13–38	–
Missing, N (%)	6 (0.6%)	0 (0%)	–
Depressed mood			
Mean (SD)	3.05 (0.88)	2.59 (0.77)	0.23 (0.56)
Range	0–4	0–4	0–3
Anhedonia			
Mean (SD)	3.63 (0.68)	2.54 (0.76)	0.26 (0.56)
Range	0–4	0–4	0–3
Sleep problems			
Mean (SD)	3.46 (2.00)	3.42 (1.77)	0.71 (0.99)
Range	0–6	0–6	0–3
Fatigue			
Mean (SD)	1.49 (0.68)	1.68 (0.55)	0.66 (0.81)
Range	0–2	0–2	0–3
Changes in appetite			
Mean (SD)	0.77 (0.66)	0.68 (0.80)	0.25 (0.62)
Range	0–2	0–2	0–3
Psychomotor changes			
Mean (SD)	1.44 (0.96)	1.42 (0.72)	0.07 (0.34)
Range	0–4	0–4	0–3
Suicidality			
Mean (SD)	1.35 (1.15)	0.94 (0.85)	0.05 (0.28)
Range	0–4	0–4	0–3

Note: MDD = Major Depressive Disorder, SD = Standard deviation, PHQ-9 = Patient Health Questionnaire-9, HAM-D = Hamilton Rating Scale for Depression.

3.1.1. Fused Graphical LASSO (FGL) estimation suggests four consistent PRS-symptom edges according to criteria 1 & 2

Using FGL estimation, we obtained networks that are visualised in Fig. 2. PRS-symptom edge bootstrapping results are displayed in Fig. 3 with PRS-PRS and symptom-symptom edge bootstrapping results shown in Supplementary Figs. 4 and 5.

As expected, nodes within the same plane displayed relatively stronger within-plane (i.e., symptom-symptom & PRS-PRS) than between-plane (i.e., PRS-symptom) associations. Among PRSs, CRP displayed associations with BMI (edge weight range across samples: 0.16–0.19) while IL-6, IL-10, and TNF-α (based on the same GWAS) were associated with each other (edge weight range across samples: 0.08–0.52). Associations of BMI and CRP with IL-6, IL-10, and TNF-α were largely absent or very small (edge weight range across samples: −0.02–0.01). Among symptoms, the largest associations were present between the core symptoms depressed mood and anhedonia (edge weight range across samples: 0.14–0.55), which is to-be-expected in clinical samples where these symptoms form the basis of the MDD diagnosis. Edge bootstrapping results in Supplementary Fig. 4 also illustrate interesting edge differences between samples that are likely arising from the diverging symptom definitions in individual samples. For instance, edges of fatigue with changes in appetite (edge weight = 0.21) and sleep problems (edge weight = 0.33) were relatively larger in the UK Biobank, assessing composite symptoms of changes in appetite and sleep problems, but substantially smaller in MARS (fatigue-changes in appetite: edge weight = 0.09; fatigue-sleep problems: edge weight = 0.10) and STAR*D (fatigue-changes in appetite: edge weight = -0.01; fatigue-sleep problems: edge weight = -0.01), assessing loss of appetite and insomnia.

Regarding PRS-symptom edges, FGL estimation surprisingly resulted in a much larger number of PRS-symptom edges in MARS and STAR*D samples compared to the UK Biobank sample with 28 (MARS), 29 (STAR*D), and 6 (UK Biobank) nonzero PRS-symptom edges. 26 (MARS), 28 (STAR*D), and 5 (UK Biobank) of these edges fulfilled criterion 1 (nonzero edges are nonzero and directionally consistent in > 50% of bootstraps). Although the difference between samples could have resulted from network differences of clinical versus general population-based samples, it may also reflect some degree of inconsistency or even noise as edge estimates often exhibited unstable directions of association in clinical samples (see Table 2).

Applying consistency criterion 2 (consistency of results across samples), we observed replicable edges of the CRP PRS with anhedonia (negative edge weight), changes in appetite, and fatigue as well as of the TNF-α PRS with fatigue; these edges were manually unfaded in Fig. 2. It is important to note that the edge between the CRP PRS and changes in appetite has a diverging valence in individual samples; in MARS and STAR*D (assessing loss of appetite) the edge weight was negative and in the UK Biobank study (assessing changes in appetite) the edge weight was positive.

3.1.2. Unregularized model search estimation suggests three consistent PRS-symptom edges according to criterion 3

Using unregularized model search estimation, we again observed networks with relatively larger within-plane (i.e., PRS-PRS & symptom-symptom) than between-plane (i.e., PRS-symptom) edges. Networks were comparable to FGL estimation, but generally sparser than those using FGL estimation; see network graphs in Supplementary Fig. 6 and

Table 2
PRS-symptom edge consistency criteria (C) across network analyses.

	MARS		STAR*D		UK Biobank		
PRS-symptom edges	FGL (C1)	Model search (C3)	FGL (C1)	Model search (C3)	FGL (C1)	Model search (C3)	FGL consistency (C2)
CRP							
Anhedonia	−0.016 (67%)		−0.043 (95%)		−0.002 (60%)		Yes
Depressed mood	−0.009 (57%)		0.045 (94%)				
Sleep problems*	−0.02 (75%)		0.031 (84%)				
Fatigue	0.053 (98%)		0.025 (79%)		0.011 (100%)		Yes
Changes in appetite*	−0.034 (89%)		−0.043 (94%)		0.003 (91%)	0.013 (73%)	Yes
Psychomotor changes	0.039 (91%)		0.001 (53%)				
Suicidality	−0.02 (74%)		−0.038 (88%)				
IL-6							
Anhedonia	−0.047 (97%)						
Depressed mood	−0.001 (52%)						
Sleep problems*			−0.02 (82%)				
Fatigue	0.012 (72%)						
Changes in appetite*	−0.032 (87%)		0.032 (85%)				
Psychomotor changes							
Suicidality	0.053 (95%)		−0.029 (83%)				
IL-10							
Anhedonia	0.008 (67%)		−0.005 (66%)				
Depressed mood			−0.002 (52%)				
Sleep problems*			0.014 (72%)				
Fatigue			0.007 (66%)				
Changes in appetite*	0.021 (80%)						
Psychomotor changes	0.015 (66%)		0.033 (90%)				
Suicidality			0.054 (99%)				
TNF-α							
Anhedonia	0.054 (96%)		0.013 (66%)				
Depressed mood	−0.017 (66%)		−0.017 (75%)				
Sleep problems*	−0.005 (57%)		0.005 (68%)				
Fatigue	0.016 (65%)		0.032 (91%)		0.002 (58%)		Yes
Changes in appetite*	−0.015 (70%)		0.023 (75%)				
Psychomotor changes	0.008 (63%)		0.008 (59%)				
Suicidality			0.047 (94%)				
BMI							
Anhedonia	0.036 (91%)		0.033 (86%)			−0.010 (63%)	
Depressed mood							
Sleep problems*	0.06 (99%)		0.055 (97%)				
Fatigue	−0.016 (74%)						
Changes in appetite*			−0.031 (87%)		0.054 (100%)	0.066 (100%)	
Psychomotor changes	0.003 (55%)		−0.023 (83%)				
Suicidality	0.021 (75%)		0.04 (91%)				

Note: Cell values reflect edge weights (i.e., partial correlation coefficients) and the percentage of 500 bootstrap estimations that edges were present. Estimates are restricted to those edges, for which > 50% of bootstrapped samples were non-zero and directionally consistent (i.e., criteria 1 & 3). *Changes in appetite and sleep problems are measured as composite symptoms in UK Biobank, but as loss of appetite and insomnia in MARS and STAR*D samples.

bootstrapping results in Supplementary Figs. 7–9.

Regarding PRS-symptom edges, only three edges were estimated as nonzero, which were all observed in the UK Biobank sample and fulfilled consistency criterion 3 (nonzero edges are also nonzero and directionally consistent in > 50% of bootstraps); these edges have been manually unfaded in Supplementary Fig. 6. The specific PRS-symptom edges were between the BMI PRS and changes in appetite and anhedonia and between the CRP PRS and changes in appetite. Comparing these edges to FGL estimation, the edge of the CRP PRS with changes in appetite replicated one of the edges fulfilling consistency criteria 1 and 2 while the two edges observed for the BMI PRS were only fulfilling consistency criterion 1 (presence in FGL estimation and > 50% of bootstraps). Moreover, the BMI PRS association with anhedonia was negative using unregularized model search estimation, but positive using FGL estimation.

3.2. Node predictability

Average node predictability was similar across samples for PRS nodes with 16% (UK Biobank), 17% (MARS), and 19% (STAR*D) of variance explained by all other nodes in the network. Contrary to this, average node predictability for symptom nodes differed with 38% of variance explained by all other nodes in the UK Biobank sample and only 9% in both clinical samples. These findings highlight differences in network density of symptoms in the UK Biobank (using the PHQ-9) and clinical samples (using the HAM-D).

4. Discussion

The present study investigated associations of PRSs for immunometabolic markers with depressive symptoms using a multi-plane, multi-sample network analysis approach. Based on three consistency criteria emphasising robustness and replicability of network analysis results across statistical bootstraps, samples, and estimation methods, we observed a unique association between the CRP PRS and changes in appetite that met all three consistency criteria. In addition to this association, we observed five additional PRS-symptom associations that met two consistency criteria. These included edges of the CRP PRS with anhedonia (negative association) and fatigue, the TNF-α PRS with fatigue, and the BMI PRS with anhedonia and changes in appetite. However, the BMI PRS-anhedonia association switched association direction depending on the estimation method, so may not be fully consistent despite fulfilling our consistency criteria. Due to the novelty of our analysis approach, we highlight several methodological considerations

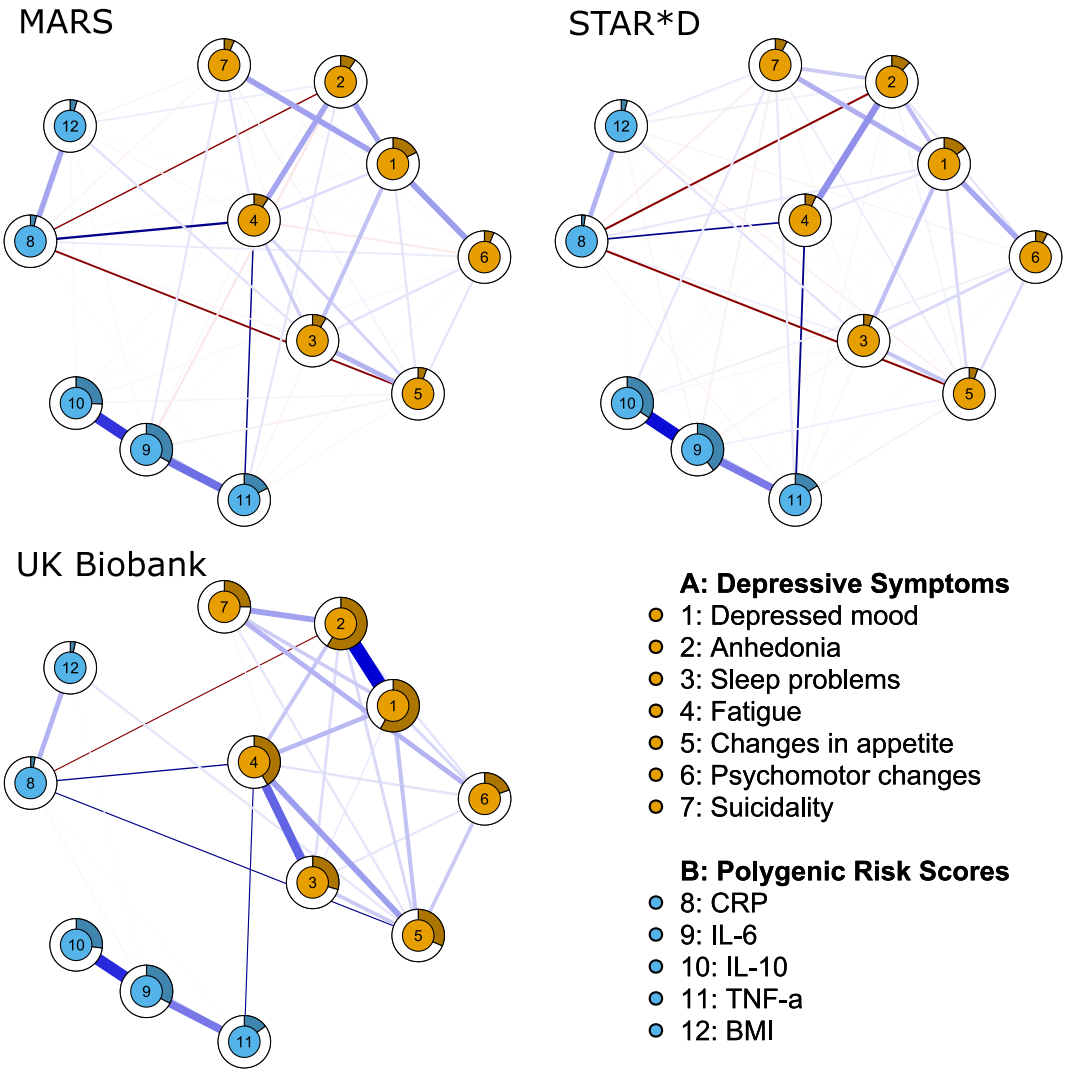

Fig. 2. Estimated FGL networks across samples. Networks are visualised with the *qgraph* package. Blue lines indicate positive and red lines negative associations, respectively, with larger associations displayed with thicker lines. Circles around nodes display node predictability, which can be interpreted similar to explained variance. Maximum size of edge associations is 0.55. As the primary focus of this investigation was to identify consistent PRS-symptom associations, we manually unfaded edges between PRSs and symptoms if these edges met quality criteria 1 and 2 (see Table 2). Changes in appetite and sleep problems are measured as composite symptoms in UK Biobank, but as loss of appetite and insomnia in MARS and STAR*D samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

below, which we hope provides a helpful framework to the discussion of our findings afterwards.

4.1. Methodological challenges and opportunities

Combining PRSs with psychological symptom networks is a relatively recent extension of network analysis and, to our knowledge, has only been applied in one previous investigation incorporating a schizophrenia PRS into a psychotic symptom network (Isvoranu et al., 2020). Therefore, it is important to emphasise the unique challenges and opportunities of this approach.

First, as noted by Isvoranu et al. (2020), statistical power is potentially the greatest challenge of PRS-symptom network analysis. Network analysis itself requires relatively large sample sizes for psychological symptom networks (Epskamp et al., 2018; Fried and Cramer, 2017), which should be in the hundreds or thousands depending on the number of nodes in the network. Inclusion of PRSs into psychological symptom networks, and especially of potential pathomechanistic (e.g., inflammatory) rather than main illness (e.g., depression/schizophrenia) scores into these networks, aggravates the sample size requirements for network analysis as PRSs only explain a fraction of variance in the heritable component of their target phenotypes (Choi et al., 2020; Wray et al., 2020).

Second, and because PRSs only measure a fraction of variance in their target phenotype, unique associations observed in network analyses are inevitably smaller than actual target phenotype-symptom associations. Taking this study as an example, absolute sizes of CRP PRS-symptom associations were 5- to 10-fold smaller than those from a prior network investigation using serum CRP concentrations by Moriarity et al. (2020a). Therefore, PRS-symptom associations are unlikely to give meaningful insights into size of association with the target phenotype, but should, in our opinion, be interpreted based on robust presence/absence of specific associations.

Third, the large statistical power requirements and difficulty quantifying such power for a given study may lead to biased result interpretations. Absence of PRS-symptom associations could be interpreted as false negatives while presence of association may be interpreted as true positives. Such divergence in interpretation necessarily biases the literature towards hypothesis confirmation. Consequently, any associations observed in PRS-symptom network analyses

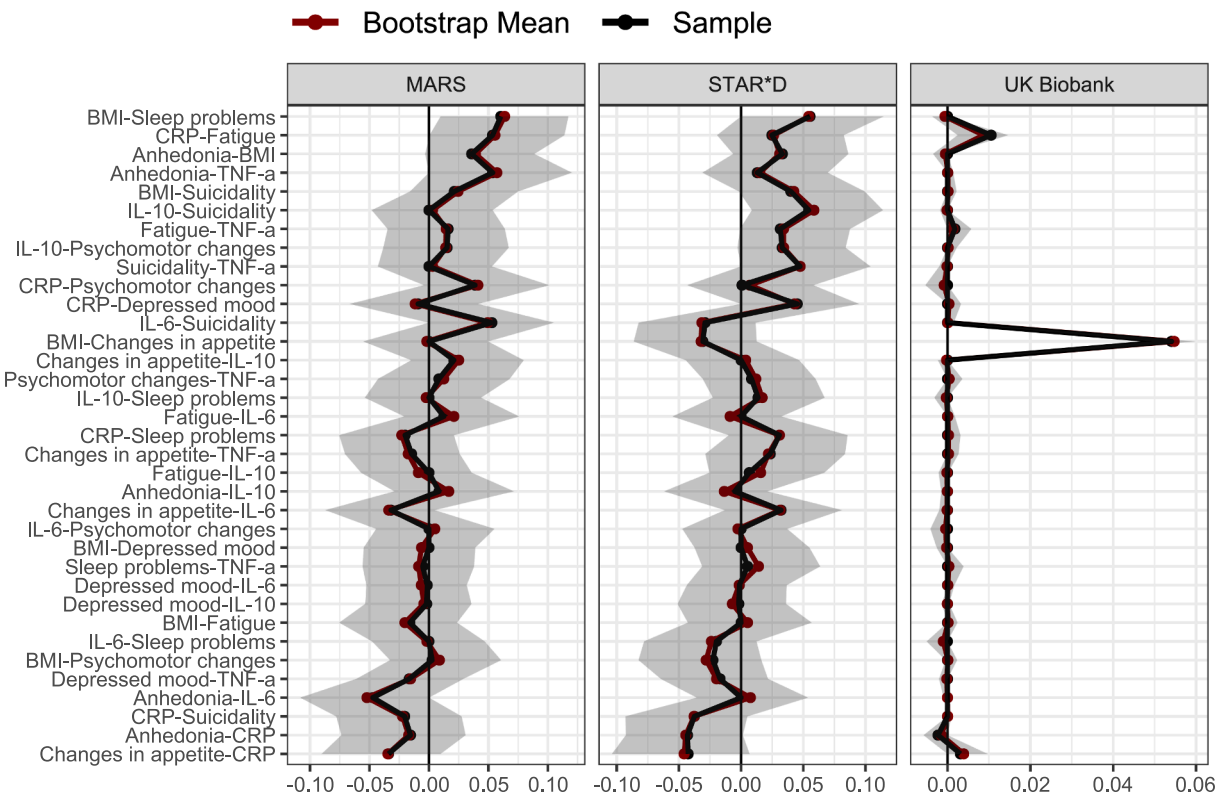

Fig. 3. Bootstrapped 95% quantile intervals of PRS-symptom edges using FGL estimation. Bootstrapped 95% quantile intervals (i.e., 95% of the distribution of raw bootstrapped edge estimates) are highlighted as shaded area for each edge. Black points indicate the raw FGL sample estimate while red points indicate the raw bootstrapped mean estimate. Edges are indicated on the y-axis and sorted by mean edge weight across samples in descending order. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

should be followed up by- and interpreted in line with- evidence from other studies, thus adhering to the recommended triangulation of evidence approach (Lawlor et al., 2017; Ohlsson and Kendler, 2019).

Despite these challenges, PRS-symptom networks also provide multiple opportunities. First, PRSs reflect estimates of genetic liability to phenotype expression, so can give an indication on the influence of lifelong predisposition to higher phenotype levels on the symptom level. In this way, PRS-symptom associations also provide an indication regarding temporality of association, which Bradford-Hill defined as one of the viewpoints for causality (Bradford Hill, 1965). It is important to note, however, that evidence for a unidirectional temporal association does not preclude bi-directionality. Moreover, PRSs combine information from a multitude of genetic variants (in our case from ~ 1 million SNPs) that are not restricted to functional SNPs, can include false positive associations (i.e., noise), and can also tag information of pleiotropic environmental confounding factors. Therefore, causal inferences should rely on separate evidence from clinical trials and/or more focused genetic approaches such as Mendelian randomisation studies (Lawlor et al., 2008).

Second, the PRS-symptom network analysis approach allows the concurrent investigation of multiple immuno-metabolic markers with multiple symptoms. Thereby, immunological and clinical complexity is addressed concurrently, which is an advantage to previous investigations. Furthermore, network analyses usually estimate partial/unique associations, so any emerging associations could suggest direct causal paths from PRS phenotypes to individual symptoms, so may pinpoint so-called 'bridge symptoms' that act as etiological docking sites of risk effects on the symptom plane.

Third, large-scale population-based or patient cohort studies, commonly used in network analysis, often do not have detailed immunophenotyping data available. If at all, studies mostly have data available for serum CRP, but rarely for more specific cytokines. Conversely, the advent of large GWAS investigations has produced a substantial amount of large cohort databases with in-depth genotyping and phenotyping information. Combining such databases with GWAS summary statistics from more focused investigations, such as on individual cytokines (Ahola-Olli et al., 2017), enables the investigation of a diverse range of immunopsychiatric research questions.

Fourth, PRS-symptom networks could be extended, for instance, by adding serum inflammatory markers to these networks, which could provide additional insights into associations between genetic/lifetime predisposition to, and acute levels of, immuno-metabolic markers with individual symptoms.

4.2. *Associations of immuno-metabolic markers with depressive symptoms*

Network analysis results showed consistent associations of the CRP PRS with changes in appetite, which was the only association that fulfilled all of our quality criteria. The BMI PRS showed similar associations with changes in appetite, but only fulfilled two quality criteria. Importantly, both of these associations were positive in the UK Biobank sample, which assessed changes in appetite, and negative in MARS and STAR*D samples, which assessed loss of appetite. Previous studies reporting results from cross-sectional, longitudinal, genetic correlation, PRS, and Mendelian randomisation analyses have also consistently reported associations of CRP/BMI with changes in appetite (Fried et al., 2019; Jokela et al., 2016; Kappelmann et al., 2021; Moriarity et al., 2020a). Importantly, whenever studies disaggregated appetite symptoms into decreased versus increased appetite, associations of CRP/BMI were specific to increased appetite (Lamers et al., 2018; Milaneschi et al., 2021b, 2021a, 2017b; Pistis et al., 2021; Simmons et al., 2018). In light of these findings, our results provide indirect support for an

immune-metabolic contribution to increased appetite specifically.

In addition to these PRS associations with changes in appetite, we also observed associations of higher CRP PRS with lower anhedonia and greater fatigue and of higher TNF-α PRS with greater fatigue. Fatigue in particular has long been considered to have a neuroimmune basis (Dantzer et al., 2014), is common across other medical illnesses characterised by chronic inflammation, and has been reliably associated with inflammatory markers in previous studies including two network investigations (Fried et al., 2019; Jokela et al., 2016; Lamers et al., 2020; Moriarity et al., 2020a; van Eeden et al., 2020; White et al., 2017). While there have also been some studies suggesting associations of inflammatory markers with anhedonia (Köhler-Forsberg et al., 2017; van Eeden et al., 2020), it is important to note that associations of the CRP PRS with anhedonia observed in the present report were negative, so do not offer straightforward replication of these findings. Nonetheless, we have recently shown in Mendelian randomisation analyses that BMI could be a potential causal factor for both fatigue and anhedonia (Kappelmann et al., 2021), so continued investigation of these symptoms is warranted.

Together, our findings add to the notion of an immuno-metabolic subtype of depression characterised by neurovegetative symptoms of changes in appetite and fatigue (Dantzer et al., 2008; Milaneschi et al., 2020). We also expand upon previous work by showing that genetic/lifetime predisposition to higher inflammation and metabolic dysregulation increases risk for depression and, based on network analysis results, these etiological factors may specifically confer their risk on the broader depression syndrome through symptoms such as changes in appetite and fatigue. These results can inform the design of clinical trials of anti-inflammatory approaches and metabolic interventions by specifically selecting patients with an atypical, neurovegetative symptom presentation. As clinical trials for immune-modulating drugs are currently still characterised by relatively small sample sizes (Husain et al., 2020; Khandaker et al., 2018; McIntyre et al., 2019; Nettis et al., 2021; Raison et al., 2013), it may be worthwhile to pilot new interventions with neurovegetative symptoms/phenotypes as outcome variables. This might increase statistical power and sensitivity to detect effects for these proof-of-concept trials and could then be followed up by larger trials testing broader clinical efficacy measures.

4.3. Strengths and limitations

Strength of this study include availability of large general population-based and patient samples (maximising replicability and generalisability), polygenic definition of immuno-metabolic risk variables (indexing lifetime predisposition to higher immuno-metabolic marker levels), and application of network analysis (addressing immunological and clinical complexity concurrently). We have addressed some of the more general limitations of combined PRS-symptom network analysis above, but there are three more specific limitations that warrant mentioning.

First, data used in the current study included inpatients, outpatients, and individuals from the general population and was based on different scales to measure depressive symptoms. Depressive symptom structure varies between acutely ill patients versus those in remission (van Borkulo et al., 2015), which may have influenced PRS-symptom associations. Moreover, two of the seven symptoms used in the present report only overlap partially; the UK Biobank study includes conflated items on sleep problems and changes in appetite while MARS and STAR*D include items on insomnia and loss of appetite, respectively. This difference may explain some of the inconsistencies observed in the current report such as the diverging valence of edge estimates between CRP and changes in appetite. However, this may have also reduced statistical power to detect associations. Study questionnaires also differed regarding the method of assessment as the HAM-D is observer-rated and the PHQ-9 self-reported. By definition, inflammation-symptom research is affected from modality-specific measurement variability (Moriarity and Alloy, 2021) and in our study this is aggravated through the added variability unique to the method of symptom assessment (Möller, 2000). Future studies would benefit from inclusion of studies with the same questionnaire and disaggregated symptom measures.

Second, the combination of clinical and general population samples poses unique challenges. The application of a clinical depression measure in the UK Biobank study could have resulted in potential floor effects for some symptoms while specific selection of MDD patients into MARS and STAR*D studies could have resulted in ceiling effects for core symptoms of depressed mood and anhedonia as these are required for a diagnosis. Selection of clinical populations in network studies can also result in Berkson/collider bias (de Ron et al., 2019), which can induce negative correlations. This again warrants replication of our results in independent samples.

Third, PRSs are based on GWASs with highly diverging samples sizes as a large number of individuals were included in the GWAS for BMI and CRP (>200 thousand individuals) and smaller numbers of individuals (~8 thousand individuals) for IL-6, IL-10, and TNF-α. Consistency of effect sizes following the PRS-CS approach was also larger for CRP and BMI as compared to IL-6, IL-10, and TNF-α. This is likely to have shifted the balance of statistical power towards detection of PRS-symptom associations to BMI and CRP rather than IL-6, IL-10, and TNF-α. Therefore, our findings require replication once larger individual cytokine GWASs become available.

5. Conclusion

The present investigation studied associations between four major pro- and anti-inflammatory markers, BMI, and depressive symptoms by applying network analysis across one large general population and two patient samples. Defining immuno-metabolic markers using polygenic risk scores expanded previous reports by suggesting direct associations of genetic/lifetime predisposition to immune-metabolic markers with depressive symptoms and provided evidence for temporality of association. Despite methodological restrictions of the presented approach, we observed associations of polygenic risk for CRP with changes in appetite and fatigue, for TNF-α with fatigue, and similar associations for BMI. These findings align with recent conceptualisations of an immuno-metabolic subgroup of depressed patients characterised by atypical, neurovegetative symptom profiles. Results can inform future clinical trials of anti-inflammatory approaches by prioritising these patients for selection into clinical trials.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbi.2021.03.024.

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Appendix A: Association of inflammation with depression and anxiety symptoms

Summary

To establish diagnostic and symptom specificity of inflammation as a risk factor in affective disorders, the objective of this third study was to test if serum CRP and IL-6 were consistently associated with all depressive and anxiety symptoms, to test the relevance of symptom direction of composite depressive symptoms, and to evaluate potential causal effects of CRP and IL-6 on depressive and anxiety symptoms using 1-sample and 2-sample MR. To this end, data from UK Biobank (N=147,478) and Netherlands Study of Depression and Anxiety (NESDA; N=2,905) cohorts was analysed. In multiple linear regression, associations of serum CRP and IL-6 with specific depressive and anxiety symptoms were tested using sequential adjustment for potential confounding variables including age, sex, and socioeconomic status (Model 1), additional adjustment of smoking, alcohol consumption, physical activity and type 2 diabetes or cardiovascular disease (Model 2), and additional adjustment of BMI (Model 3). In NESDA, symptom direction of these associations was further investigated for composite symptoms of changes in appetite, sleeping problems, and psychomotor changes. Finally, 1-sample and 2-sample MR analysis was applied to test potential causal associations using genetic variants indexing CRP and IL-6 based on associations with serum CRP concentrations in the UK Biobank or estimates from discovery GWAS, respectively.

Meta-analysis of associations across UK Biobank and NESDA cohorts suggested CRP was associated with depressive symptoms of depressed mood, fatigue, sleeping problems, and changes in appetite following multiple comparison correction and adjustment for sociodemographic and lifestyle confounders (Model 2), but the association with changes in appetite fully attenuated with additional adjustment for BMI. Extended analyses in NESDA showed that associations of CRP were specific to hypersomnia rather than symptoms indexing aspects of insomnia. They were also specific to increased appetite rather than decreased appetite. Findings for IL-6 in NESDA further suggested associations of higher IL-6 with hypersomnia, fatigue, decreased appetite and anhedonia. Finally, 1-sample and 2-sample MR analyses suggested potential causal associations of higher CRP with lower suicidal ideation and lower cognitive problems and of higher IL-6 activity with greater fatigue and sleeping problems following multiple comparison correction. Taken together, these findings provide consistent evidence implicating the IL-6/IL-6R pathway in the aetiology of depressive symptoms of fatigue and sleeping difficulties. They also

highlight the importance of considering metabolic dysregulation in the context of inflammation and of considering symptom direction for symptoms of changes in appetite and sleeping problems. These results provide a more fine-grained picture implicating inflammation specifically in the aetiology of so-called atypical symptoms of depression, which emphasises the benefit of studying potential anti-inflammatory and lifestyle interventions targeting this group.

Contributions and reference

The study "Association of Inflammation with Depression and Anxiety: Evidence for Symptom-Specificity and Potential Causality from UK Biobank and NESDA Cohort" was posted as a preprint on medRxiv in January 2021 and has been accepted by Molecular Psychiatry in June 2021. YM and NK were responsible for writing of the original draft; YM, NK, ZY, FL, SM, PBJ, SB, BWJHP, and GMK for reviewing and editing the manuscript; YM, NK, ZY, FL, BWJHP, and GMK for conceptualisation of the study; NK for visualisations; YM, NK, ZY, and SM for data preparation and analysis; and PBJ, SB, BWJHP, and GMK for supervision.

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Note

The manuscript presented on the subsequent pages represents the most recent version of the manuscript in June 2021 and as accepted in Molecular Psychiatry. It is identical in content, but has been formatted to align with the format of this thesis.

Association of Inflammation with Depression and Anxiety: Evidence for Symptom-Specificity and Potential Causality from UK Biobank and NESDA Cohorts

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INTRODUCTION

A role for inflammatory dysregulation in depression has been suggested by a large body of evidence. Clinical studies have shown that a quarter of patients with hepatitis C develop a depressive episode following pro-inflammatory interferon treatment [1]. Large meta-analyses report cross-sectional and longitudinal associations between inflammatory markers - such as C-Reactive Protein (CRP) and Interleukin 6 (IL-6) - and depression [2–7]. Indeed, inflammation is present in about a quarter of depressed patients as evidenced by elevated CRP levels [8]. However, there are key outstanding questions regarding symptom specificity and potential causality of association.

Previous studies have mainly used composite measures of depression, but it is a phenotypically heterogenous syndrome. Depression is also highly comorbid with anxiety, but

studies of inflammation and anxiety symptoms are scarce [9]. It is possible that inflammation is relevant for some but not all affective symptoms. Higher inflammatory marker levels have been reported to be mainly associated with anhedonia and neurovegetative symptoms such as fatigue, appetite and sleep alterations [10–16]. However, assessments in previous research often conflated divergent alterations in neurovegetative symptoms (e.g. increased vs decreased appetite). Emerging evidence suggests that inflammatory and metabolic alterations map more consistently onto "atypical" energy-related symptoms, particularly increased sleep, appetite/weight, fatigue and leaden paralysis [17–19]. Inflammation is unlikely to be relevant for all cases of depression. A symptom-based approach may provide insights into mechanisms of inflammation-related depression and could help inform patient selection in immunotherapy trials.

In addition to symptom-specificity, another key issue is causality of association. Mendelian Randomization (MR) is an approach to evaluate potential causality, which uses genetic variants as proxy instruments (unrelated to confounding variables based on Mendel's law of random allele segregation) to test exposure-outcome associations [20]. A recent MR study of depressive symptoms indicated that IL-6 signalling could be causally linked with suicidality [21]. However, this study did not triangulate MR results using phenotypic association analyses on serum inflammatory markers and did not investigate anxiety symptoms or particular direction of change (increase vs decrease) in neurovegetative symptoms specifically. MR studies of depressive and anxiety symptoms including more granular information on direction of symptom change is required to gain greater insights into the potential role of inflammation in these disorders.

We have examined specificity and potential causality of associations for CRP and IL-6 with depressive and anxiety symptoms using large-scale data from two well-established European cohorts, UK Biobank (UKB) and Netherlands Study of Depression and Anxiety (NESDA). In addition to testing symptom-level associations for CRP in two cohorts, we have carried out further analysis in the NESDA cohort using: (1) IL-6 levels as exposure; and (2) granular information on direction of change for particular symptoms (e.g., sleep problems, appetite alterations) as outcomes in relation to IL-6 and CRP. Furthermore, we have carried out one- and two-sample MR analyses to test whether associations of IL-6 and CRP with specific depressive and anxiety symptoms are likely to be causal using genetic variants regulating levels/activity of these inflammatory markers as instrumental variables.

METHODS

Study cohorts

The present study utilised data from the UK Biobank (UKB) study [22], a population-based cohort comprising 502,524 UK residents aged 40-69 years, and the Netherlands Study of Depression and Anxiety (NESDA) [23], an ongoing cohort study of 2,981 participants aged 18-65 years with current or past depressive and/or anxiety disorder and healthy controls. Detailed descriptions of study cohorts are available as Supplementary Methods.

Briefly, we included up to 147,478 participants from the UKB sample with data on CRP and depressive/anxiety symptoms. For Mendelian Randomisation analysis, a sample of unrelated individuals with European ancestry and genotype information was used including up to 325,441 participants to estimate single-nucleotide polymorphism (SNP)-exposure associations with CRP for one-sample MR analysis and up to 111,572 participants to estimate SNP-outcome associations. From the NESDA sample, 2,905 participants with complete data at baseline on inflammatory markers and depressive/anxiety symptoms were selected. Biomarkers and symptoms were assessed again at 2-year and 6-year follow-up, totalling ~7,000 observations.

The UKB study was approved by the UK Biobank's research ethics committee and Human Tissue Authority research tissue bank. The current analysis was approved under project no. 26999. The NESDA research protocol was approved by the ethical committee of participating universities. Participants from both cohorts provided informed consent.

Depressive and anxiety symptoms

In UKB nine depressive and seven anxiety symptoms were assessed using Patient Health Questionnaire (PHQ)-9 and Generalized Anxiety Disorder (GAD)-7 questionnaires, respectively [24]. We identified similar items from NESDA that were assessed by different questionnaires (i.e., Inventory of Depressive Symptomatology [IDS-SR_{30}], Beck Anxiety Inventory [BAI] & Penn State Worry Questionnaire [PSWQ]) at baseline, 2- and 6-year follow-up. Items coding similar domains (e.g., anhedonia assessed as lack of "general interest" and "capacity for pleasure") and items coding specific neurovegetative symptoms (e.g., sleeping problems separately measured as "increased" or "decreased" sleep) were conflated to align these with UKB data for ease of comparison. All symptoms were binarised to reflect a measure of any versus no symptom endorsement. For depressive symptoms we also created two summary scores, "psychological" and "somatic", based on a 2-factor model based on reported genetic covariance among nine PHQ-9 symptoms in UKB [25].

In extended NESDA analyses, items measuring neurovegetative symptoms were left disaggregated coding for specific alterations. Details on questionnaires, items and coding are in Supplementary Methods and Supplementary Table 1.

Inflammatory markers

In both UKB and NESDA, circulating CRP levels were measured using high-sensitivity assays. In NESDA, circulating IL-6 levels were additionally available, and both inflammatory markers were assessed at baseline, 2- and 6-year follow-up which were modelled in repeated-measurement analyses. Details on blood sampling and technical assay features are described in Supplementary Methods.

Covariates

The same set of covariates was considered in both cohorts, which included age, sex, socioeconomic status (SES), smoking (current/former/never), quantity of alcohol consumption, type and time spent in physical activity, lifetime history of type two diabetes (T2D) and cardiovascular diseases (CVD), and body mass index (BMI). SES was measured via the Townsend Deprivation Index (a composite score of deprivation derived from national census data [26]) in UKB, and as years of education in NESDA. All covariates in NESDA were measured at baseline. Covariate measurements and their distribution are described in Supplementary Methods and Supplementary Tables 2 and 3.

Statistical Analysis

Cross-cohort analyses of associations between CRP and depressive/anxiety symptoms

Associations between CRP levels and depressive/anxiety symptoms were estimated by regressing individual symptoms and summary scores on log-transformed values of CRP. Details of statistical models are given in Supplementary Methods. In order to explore the impact of covariates on the association between CRP and symptoms, estimates were adjusted for age, sex and SES (Model 1), additionally adjusted for smoking, alcohol consumption, physical activity and T2D/CVD (Model 2), and additionally adjusted for BMI (Model 3).

Cohort-specific estimates were pooled using random-effects meta-analysis with the DerSimonian and Laird method [27]. For each model, False-Discovery Rate (FDR) q-values were calculated taking into account testing across 16 symptoms and 2 summary scales.

Extended analyses using IL-6 levels and additional symptoms in NESDA

Items measuring neurovegetative (appetite, sleep and psychomotor) symptoms were disaggregated in order to estimate associations between specific alterations (e.g., increase vs decrease) and inflammation. We also used (log)IL-6 levels as exposure, not

measured in UKB. For each model, FDR q-values were calculated accounting for testing across 21 symptoms and two summary scales.

Mendelian Randomization analyses

Instrument selection

We used multiple genetic instruments for CRP [28, 29], based on SNPs in the *CRP* gene associated with serum CRP concentrations, and for IL-6 [28, 30, 31], based on SNPs in the *IL-6R* gene associated either with serum CRP concentrations (as downstream readout of IL-6) or with serum IL-6 concentrations. Further details are provided as Supplementary Methods and in Supplementary Table 4.

MR analysis

Availability of CRP concentrations in the UKB sample allowed both one-sample and two-sample MR analyses with SNP-exposure estimates obtained from original reports (two-sample MR) or by regressing CRP on SNPs in UKB (one-sample MR). SNP-outcome estimates were all obtained by regressing outcome phenotypes on SNPs. Regression analyses were controlled for 20 genotype principal components, age, age^2, sex, and age*sex. We performed standard variant harmonisation procedures on obtained estimates [32]. As main analysis, we used fixed-effects inverse variance weighted (IVW) meta-analysis per exposure-outcome combination or Wald ratio estimation for the single-SNP IL-6 instrument [30]. Potential horizontal pleiotropy was evaluated using Cochrane's Q statistic [33]. Further details are noted in Supplementary Methods.

RESULTS

Table A1 shows main variables including symptom endorsement and inflammatory marker levels in UKB and NESDA cohorts. See Supplementary Table 5 and Supplementary Figures 1 & 2 for main variables and CRP and IL-6 levels across NESDA assessment waves.

Table A1. Main variables of interest in the two cohorts

	UK Biobank	NESDA
	N = 143,465	*N = 2,905*
Sociodemographics		
Age *years (mean ± SD)*	55.9 (7.7)	41.9 (13.1)
Sex (F) *(%)*	56.2	66.5
Symptom endorsement - Depression (%)		
D.1 Anhedonia	18.5	13.9
D.2 Depressed mood	21.9	21.6
D.3 Sleep problem	48.7	25.9
D.4 Fatigues	49.9	36.5
D.5 Appetite change	18.2	7.7
D.6 Feelings of inadequacy	19.2	24.2
D.7 Cognitive problems	17.9	21.9
D.8 Psychomotor change	5.5	18.1
D.9 Suicidal ideation	4.3	12.2
Symptom summary scales		
Psychological symptoms *(mean ± SD)*	0.74 (1.51)	0.721 (1.04)
Somatic symptoms *(mean ± SD)*	1.65 (2.03)	1.09 (1.30)
Symptom endorsement - Anxiety (%)		
A.1 Anxiety	28.0	24.5
A.2 Worrying control	23.4	24.8
A.3 Generalized worrying	31.6	31.5
A.4 Lack of relaxation	28.3	32.3
A.5 Restlessness	11.8	25.7
A.6 Irritability	27.2	21.0
A.7 Foreboding	16.6	20.4
Inflammatory markers		
CRP *(mg/L) (median, IQR)*	1.15 (0.58-2.37)	1.22 (054 - 3.00)
IL-6 *(pg/mL) (median, IQR)*	NA	0.75 (0.49 - 1.25)

Note: UK Biobank sample size reflects all individuals with complete symptom and CRP data. For NESDA baseline values are reported; measures at 2- and 6-year follow-up are reported in Supplementary Table 5 and Supplementary Figures 1 & 2. NA=not applicable.

Association between CRP and depressive/anxiety symptoms in UKB and NESDA

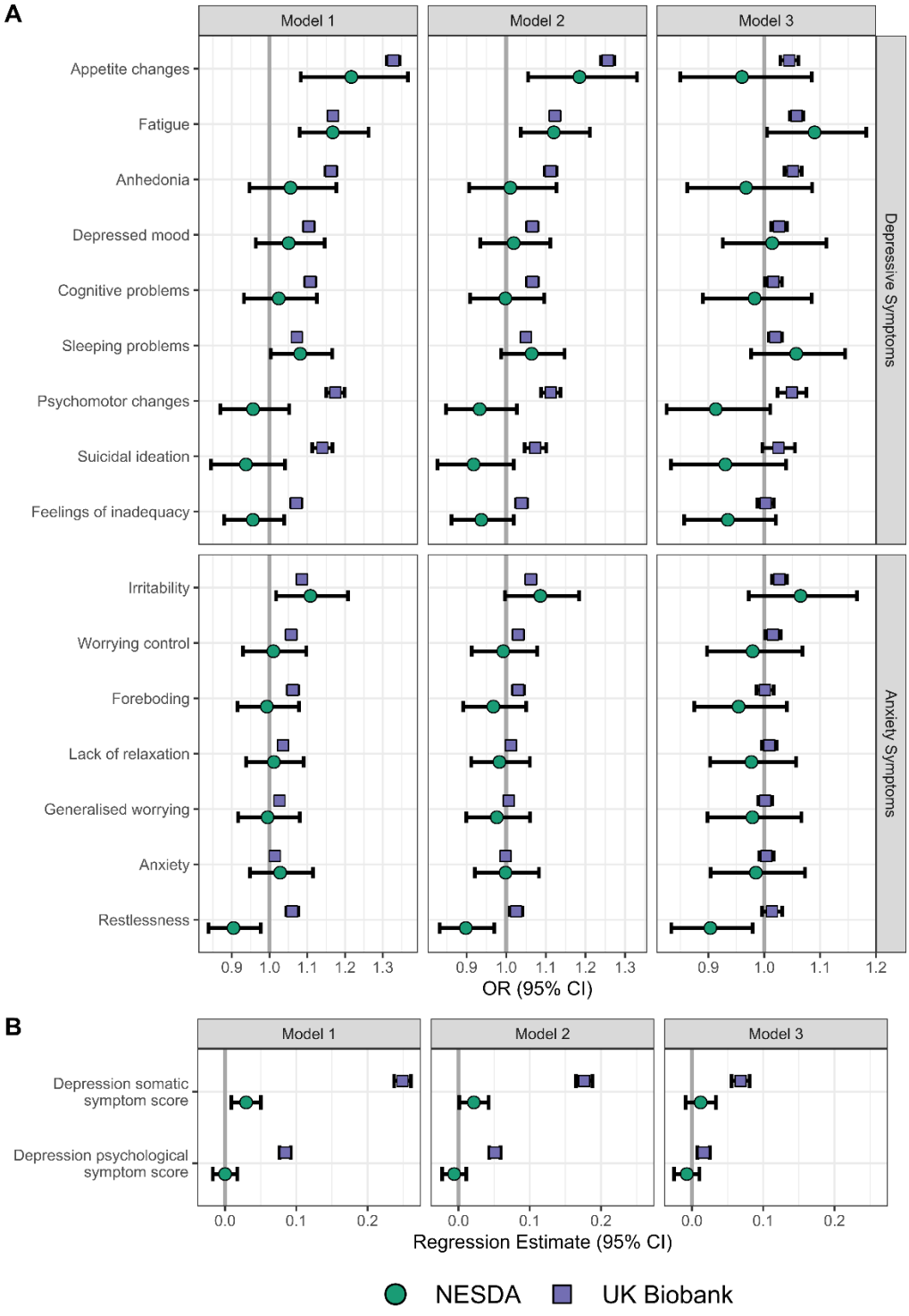

Figure A1. Association estimates of CRP with depressive and anxiety symptoms from UKB and NESDA cohorts. Association estimates are shown with individual depressive and anxiety symptoms (A) and depressive summary scores (B). Models have been adjusted for age, sex and SES (Model 1), additionally adjusted for smoking, alcohol consumption, physical activity and T2D/CVD (Model 2), and additionally adjusted for BMI (Model 3).

Table A2. Pooled Association Results between CRP and Depressive/Anxiety Symptoms

	Model 1			Model 2			Model 3		
Symptom	**OR (95% CI)**	**P**	**Q**	**OR (95% CI)**	**P**	**Q**	**OR (95% CI)**	**P**	**Q**
Depressive symptoms									
Anhedonia	1.13 (1.03-1.23)	0.01	0.02	1.08 (0.99-1.18)	0.101	0.203	1.03 (0.96-1.11)	0.423	0.789
Depressed mood	1.1 (1.07-1.13)	<0.001	<0.001	1.06 (1.05-1.08)	<0.001	<0.001	1.03 (1.01-1.04)	<0.001	0.002
Sleeping problems	1.07 (1.06-1.08)	<0.001	<0.001	1.05 (1.04-1.06)	<0.001	<0.001	1.02 (1.01-1.03)	<0.001	0.003
Fatigue	1.17 (1.15-1.18)	<0.001	<0.001	1.12 (1.11-1.14)	<0.001	<0.001	1.06 (1.05-1.07)	<0.001	<0.001
Appetite changes	1.3 (1.2-1.4)	<0.001	<0.001	1.25 (1.23-1.28)	<0.001	<0.001	1.02 (0.96-1.1)	0.499	0.789
Feelings of inadequacy	1.02 (0.91-1.14)	0.734	0.777	1 (0.9-1.1)	0.925	0.979	0.98 (0.92-1.05)	0.564	0.789
Cognitive problems	1.08 (1.01-1.16)	0.034	0.055	1.05 (0.99-1.11)	0.092	0.203	1.02 (1-1.03)	0.043	0.128
Psychomotor changes	1.07 (0.87-1.3)	0.537	0.644	1.03 (0.86-1.22)	0.778	0.875	0.99 (0.86-1.13)	0.859	0.859
Suicidal ideation	1.04 (0.86-1.26)	0.683	0.768	1 (0.86-1.17)	0.991	0.991	0.99 (0.91-1.09)	0.856	0.859
Anxiety symptoms									
Anxiety	1.01 (1-1.03)	0.012	0.022	1 (0.99-1.01)	0.758	0.875	1 (0.99-1.02)	0.606	0.789
Worrying control	1.05 (1.03-1.08)	<0.001	<0.001	1.03 (1.02-1.04)	<0.001	<0.001	1.01 (1-1.03)	0.033	0.12
Generalised worrying	1.03 (1.01-1.04)	<0.001	<0.001	1.01 (0.99-1.02)	0.309	0.505	1 (0.99-1.01)	0.833	0.859
Lack of relaxation	1.04 (1.02-1.05)	<0.001	<0.001	1.01 (1-1.02)	0.057	0.147	1.01 (1-1.02)	0.206	0.464
Restlessness	0.98 (0.84-1.15)	0.834	0.834	0.96 (0.85-1.1)	0.586	0.812	0.96 (0.86-1.08)	0.52	0.789
Irritability	1.09 (1.07-1.1)	<0.001	<0.001	1.06 (1.05-1.08)	<0.001	<0.001	1.03 (1.01-1.04)	<0.001	<0.001
Foreboding	1.04 (0.98-1.1)	0.21	0.29	1.01 (0.96-1.07)	0.677	0.871	1 (0.97-1.02)	0.812	0.859

Depressive symptom score	**β (SE)**	**P**	**Q**	**β (SE)**	**P**	**Q**	**β (SE)**	**P**	**Q**
Psychological symptoms	0.043 (0.042)	0.312	0.402	0.023 (0.029)	0.42	0.63	0.006 (0.012)	0.614	0.789
Somatic symptoms	0.14 (0.11)	0.203	0.29	0.099 (0.077)	0.2	0.36	0.041 (0.028)	0.144	0.371

Note: estimates describe the association with the outcome per 1 unit increase in *(log)*CRP.

Figure A1 shows results from individual cohorts and Table A2 shows meta-analytic pooled estimates representing the associations of CRP with individual depressive/anxiety symptoms and summary scores for somatic and psychological symptoms of depression (exact numbers in Supplementary Tables 6 & 7). Based on the pooled analyses, after adjustment for sociodemographic, lifestyle and health-related factors (Model 2), higher CRP was associated with appetite change (OR=1.25, 95%CI=1.23-1.28), fatigue (OR=1.12, 95%CI=1.11-1.14), depressed mood (OR=1.06, 95%CI=1.05-1.08), and sleep problems (OR=1.05, 95%CI=1.04-1.06) among depressive symptoms, and with irritability (OR=1.06, 95%CI=1.05-1.08) and worrying control (OR=1.03, 95%CI=1.02-1.04) among anxiety symptoms. CRP was not associated with somatic and psychological symptom summary scores for depression. Evidence for associations attenuated with increasing confounder adjustment and especially after including BMI; however, associations of CRP with fatigue, sleep problems, depressed mood and irritability remained statistically significant.

Further analyses using IL-6 levels and additional symptoms in NESDA

Among symptoms identified in cross-cohort analysis, extended analyses examining (log)IL-6 levels and disaggregated neurovegetative symptoms confirmed associations with altered sleep, appetite and fatigue (Figure A2, full results Supplementary Tables 8 & 9). In particular, both higher CRP and IL-6 showed converging associations with hypersomnia (CRP OR=1.27, 95%CI=1.13-1.43; IL-6 OR=1.26, 95%CI=1.07-1.49) and fatigue (CRP OR=1.12, 95%CI=1.04-1.21; IL-6 OR=1.19, 95%CI=1.07-1.33). In contrast, divergent associations with appetite alterations emerged, with CRP linked to increased appetite (OR=1.21, 95%CI=1.08-1.35) and IL-6 linked to decreased appetite (OR=1.45, 95%CI=1.18-1.79). IL-6 but not CRP was also associated with anhedonia (OR=1.30, 95%CI=1.12-1.52). Additional adjustment for BMI did not change results substantially, but the association between CRP and increased appetite was no longer significant. In model 2, higher IL-6 was associated with depressed mood, but not after considering multiple testing. IL-6 was not associated with anxiety symptoms.

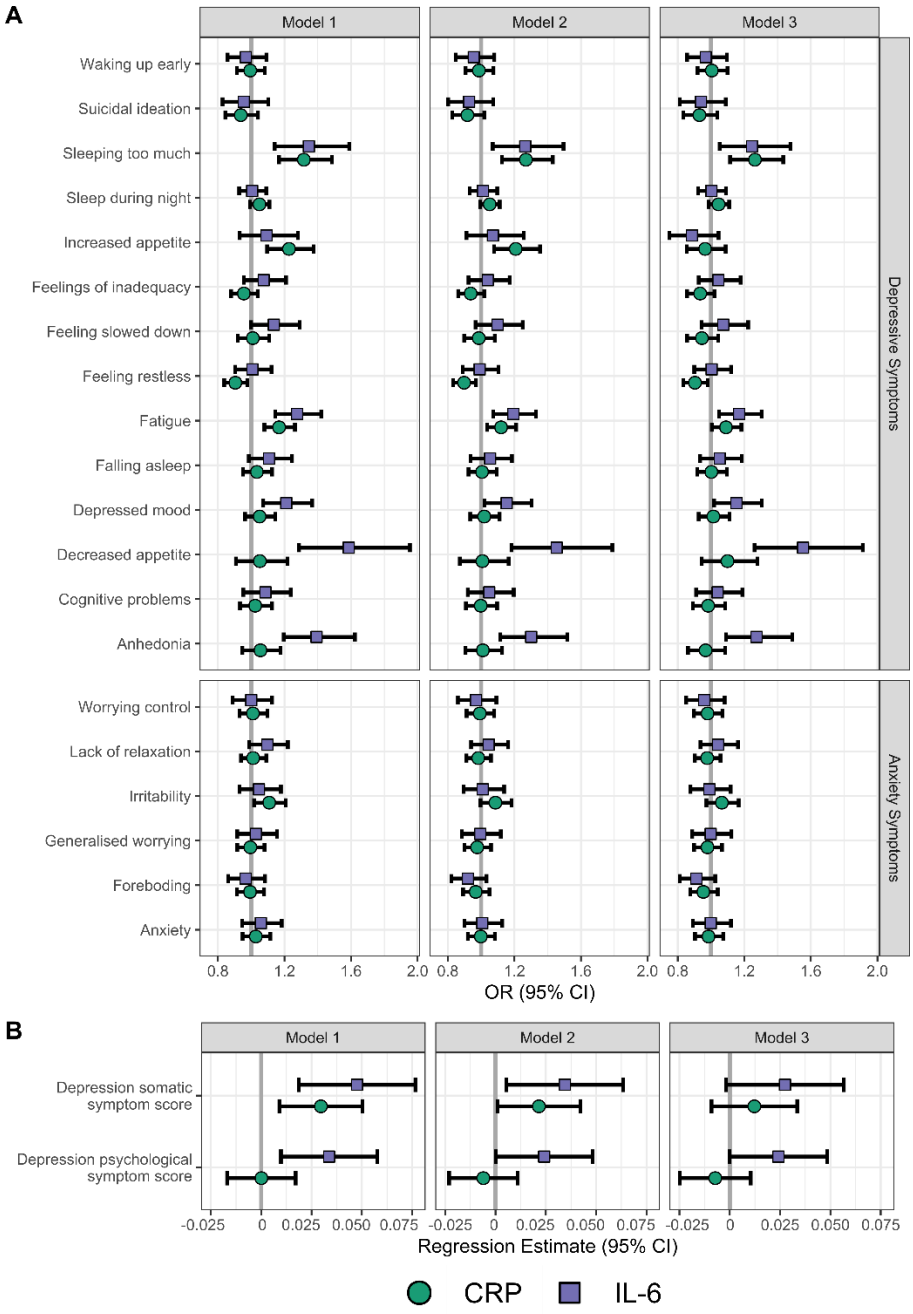

Figure A2. NESDA association estimates of CRP and IL-6 with depressive and anxiety symptoms. Association estimates are shown with individual depressive and anxiety symptoms (A) and depressive summary scores (B). Models have been adjusted for age, sex and SES (Model 1), additionally adjusted for smoking, alcohol consumption, physical activity and T2D/CVD (Model 2), and additionally adjusted for BMI (Model 3).

Results for Mendelian randomization analyses

IVW one-sample and two-sample MR results for CRP and IL-6 instruments are displayed in Figure A3 based on genetic instruments derived from Georgakis *et al.*[28] Exact numeric results for these and other instruments are provided in Supplementary Tables 10 and 11.

Overall, MR results showed that genetically predicted higher CRP levels were associated with lower risk of depressive and anxiety symptoms. After adjusting for multiple comparisons, evidence for associations remained for suicidal ideation, cognitive problems, and the psychological symptom summary score. On the other hand, MR results for IL-6 showed that genetically predicted increased IL-6 signalling was associated with increased fatigue, sleep problems and suicidality, but only associations with fatigue and sleep problems persisted after corrections for multiple testing.

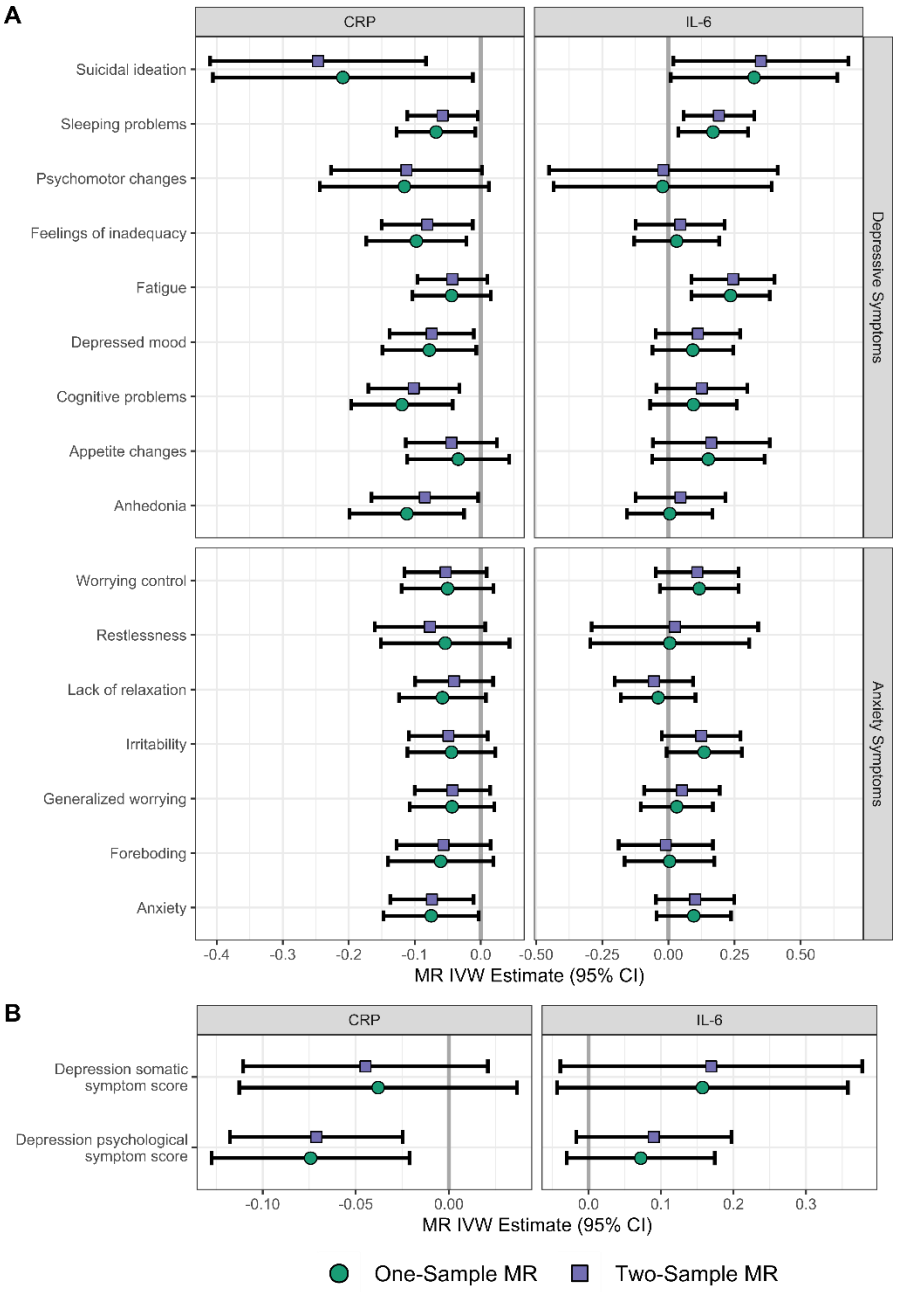

Figure A3. Mendelian randomisation results of CRP and IL-6 with depressive and anxiety symptoms. MR association estimates are shown with individual depressive and anxiety estimates (A) and depression summary scores (B). Results reflect MR IVW estimates based on Georgakis *et al.*(Georgakis et al., 2020) instruments; exact numeric values are presented in Supplementary Tables 11 & 12.

Of note, MR results for the aforementioned associations of genetically predicted CRP/IL-6 with specific depressive symptoms were directionally consistent with results from a previous report using two-sample MR and a different combination of genetic instruments for CRP and IL-6 (cf. Kappelmann *et al.*[21]).

Assessment of heterogeneity did not indicate that the aforementioned associations were likely to be due to horizontal pleiotropy except for the MR associations between lower CRP with increased suicidality (Supplementary Table 12 & 13).

DISCUSSION

Using large-scale data from two well-established cohorts, we report an extensive evaluation of the associations between inflammatory markers and individual symptoms of depression and anxiety, including cross-cohort analyses, extended phenotype analyses on symptoms assessed at more granular resolutions, and MR analysis testing potential causality. Our results provided evidence for symptom-specificity. Inflammation does not map uniformly onto all affective symptoms, but rather are more consistently associated with specific depressive symptoms of fatigue, altered appetite, sleep problems (in particular hypersomnia), and the core symptom of depressed mood as compared to other symptoms of depression and anxiety. Furthermore, we provide evidence consistent with a potentially causal role of IL-6 in fatigue and sleep alterations; please see Supplementary Table 14 for an overview of main results.

Results across different analytical models and biomarkers highlighted more consistent associations of inflammation with depressive than with anxiety symptoms. For anxiety, associations were mainly limited to CRP and irritability, a symptom also commonly present in depression. These results align with an extensive evidence-base suggesting an association between inflammation and depression, and with a more limited evidence-base indicating an association between increased CRP levels mainly in subjects with generalized anxiety disorder (GAD), including our previous work from NESDA, ALSPAC, and UK Biobank cohorts [2–5, 9, 11, 17–19, 21, 34–36]. Together, these findings support the idea that systemic inflammation could be specifically related to depressive rather than anxiety symptoms.

Our results provide evidence for further phenotype-specificity within the depression syndrome suggesting that inflammation maps specifically onto symptoms of fatigue, sleeping problems, changes in appetite, and depressed mood. These findings are consistent with previous research[10–15] including the concept of immuno-metabolic depression and

with inflammation-related "sickness behaviour" observed in human and animal studies [37, 38].

We add to this evidence-base by providing data for similar and distinct associations for IL-6 and CRP for certain symptoms, especially sleep and appetite. Only hypersomnia, but not loss of sleep, showed consistent associations with CRP and IL-6. We report an intriguing dissociation between these inflammatory markers regarding their associations with appetite. CRP and IL-6 were specifically associated with increased and decreased appetite, respectively. These findings are consistent with evidence from animal models showing that CRP directly inhibits leptin binding to its central receptors, abolishing its anorexigenic effect and disinhibiting food intake [39]. In contrast, in obese mice with leptin resistance, the central activation of IL-6 trans-signalling has been shown to suppress feeding and improve glucose tolerance [40]. Similarly, it has been previously shown that increased BMI, a major stimulus for CRP production [41], is associated with appetite alterations [21], but only with increased appetite [42]. These data shed light on the impact of obesity on the association between CRP and appetite alterations, which was fully attenuated after controlling for BMI.

The pathways linking BMI, inflammation and depression, and their role in those pathways, are particularly complex. It is known that genetic risk variants for inflammation and for depression also have a major role in BMI increase [43, 44]; this may create a configuration in which BMI is both a confounder and a collider (Supplementary Figure3), whose adjustment or lack thereof may lead to biased estimates. Nevertheless, all associations with other symptoms remained statistically significant after BMI adjustment, although relatively reduced.

Findings from MR analyses also highlight divergence between inflammatory markers. IL-6 seems to have a potential causal effect in the development of fatigue and sleep problems, in line with evidence on the role of inflammation in "sickness behaviour" [37, 38]. In contrast, CRP did not show significant MR estimates for the same symptoms, despite strong associations with circulating protein levels. These results suggest that the CRP-depressive symptom associations may represent epiphenomena emerging from common underlying factors such as metabolic dysregulation. This idea is consistent with previous evidence suggesting shared genetic liability between CRP and symptoms of fatigue, altered sleep and appetite [15, 18, 21]. Furthermore, genetically-elevated CRP levels were associated with lower risk of psychological symptoms, cognitive problems and suicidality. Similar divergent MR results have also been reported for schizophrenia and depression, suggesting a protective effect of CRP and a risk-increasing effect of soluble IL-6 receptor (IL-6R) on schizophrenia and depression risk, respectively [45, 46]. We have also

previously reported that *IL-6R* variants associated with higher serum IL-6 levels (but decreased IL-6R activity) and *CRP* variants associated with higher CRP levels are *both* associated with increased risk of depression in the UK Biobank [47]. It could be hypothesized that the genetic instrument for CRP may partially capture the activity of IL-6 classic signalling that promotes regenerative and protective responses for neuronal function [48]. Hence, IL-6 classic signalling may potentially underlie protective findings for schizophrenia and depression. Conversely, IL-6 trans-signalling, which has been implicated in chronic inflammatory conditions such as rheumatoid arthritis and is primarily responsible for the pro-inflammatory role of IL-6 [49], may be responsible for risk-increasing associations of genetic instruments for IL-6. Future analyses joinlty considering CRP and IL-6 genetic instruments may clarify the direct effect of each marker.

It is also important to investigate if immunological markers beyond CRP and IL-6 are potentially causally associated with depression, and to identify potential cellular drivers for IL-6 dysfunction. While CRP and IL-6 are commonly measured inflammatory markers, they have multiple pro- and anti-inflammatory roles such as those exerted through IL-6 classic and trans-signalling [48], which complicates inferences about specific immunological mechanisms conferring risk for depression. Case-control studies have implicated multiple other pro- and anti-inflammatory markers in depression [6] and immunophenotyping work suggests that patients exhibit increased monocyte, $CD4^+$ T cell, and neutrophil counts [50]. Mechanistically, it has been suggested that these dysregulations could contribute to depression risk via multiple processes such as kynurenine pathway activation, imbalances in T cell subsets towards a pro-inflammatory phenotype, and reduced neuroplasticity [51]. Distinguishing these processes requires further population-based studies and experimental animal and human studies including detailed immunophenotyping of patients with depression.

Finally, while MR analysis can provide evidence for causality, population genomic approaches alone are not sufficient to clarify pathophysiologic mechanisms fully and triangulation of evidence from different approaches are required, including experimental studies of immune-modulation in humans. A potential pathophysiological role for inflammation is consistent with evidence from meta-analyses showing antidepressant effects of anti-inflammatory agents [52–54]. However, some of the studies had small samples and/or were focused on patients with primary illnesses other than depression. A recent RCT [55] of anti-IL-6 monoclonal antibody sirukumab showed improvement in anhedonia but did not report significant difference in overall depression severity. Although this study used elevated CRP as entry criteria, one off measurement of CRP may not be sufficient to identify patients with persistent inflammation. A number of ongoing trials

including small proof-of-concept studies are now testing potential clinical effects of immune-modulation on depression [56, 57], especially in patients with signs of heightened and/or persistent inflammation. In future, adequately powered RCTs along with careful consideration of patient selection are required.

Strengths and Limitations

Strengths of the work include use of large samples from two European cohorts allowing replication/verification of findings, combined phenotypic and genetic analyses, and evaluation of both depressive and anxiety symptoms. A limitation was the lack of more granular data on specific vegetative depressive symptoms and IL-6 levels in UKB. Furthermore, as participants in NESDA and UK Biobank studies were relatively old and the overwhelming majority of subjects enrolled were of European ancestry, results cannot be generalized to younger populations and populations of other ancestries.

CONCLUSIONS

In the current investigation of associations of inflammation with depression and anxiety, we provide evidence for specificity at several levels. First, systemic inflammation is mainly associated with depressive rather than anxiety symptoms. Second, within depression, inflammation is particularly associated with somatic/neurovegetative symptoms such as fatigue, altered sleep, appetite as well as depressed mood and anhedonia. Third, within symptoms, IL-6 and CRP have opposing effects on appetite but similar effects on fatigue. In addition, using MR analysis we provide evidence that the IL-6/IL-6R pathway could be causally linked with fatigue and sleeping problems. The field now requires experimental studies of IL-6 modulation in humans and animals to further evaluate causality, potential pathogenic mechanisms, and to assess potential usefulness of (add-on) immunotherapies for depression.

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CONFLICTS OF INTEREST

BWJHP has received research funding (unrelated to the work reported here) from Jansen Research and Boehringer Ingelheim. The remaining authors declare that they have no conflict of interest.

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Appendix B: Role of inflammation in depression and anxiety

Summary

To identify patients with inflammation-related pathophysiology, the objectives of this fourth study were to test whether inflammation is a disorder-specific or trans-diagnostic risk factor for depression and anxiety, whether this association follows a linear dose-response pattern, whether associations differed between women and men, and if associations are likely to be causal. To meet these objectives, we used data from up to 144,890 participants of the UK Biobank study that participated in the optional follow-up mental health survey and completed questionnaires for depressive and generalised anxiety disorder (GAD) symptoms. We used multiple linear regression to assess if higher serum CRP was associated with these depressive and anxiety symptoms and binary measures of probable depression and GAD diagnoses. Bivariate probit regression and multiple regression adjusted for the respective other outcome were then applied to test evidence for potential disorder-specificity of CRP. Next, 1-sample and 2-sample MR analysis was applied to test potential causality of CRP and IL-6 on these outcomes. Finally, sex-stratified analyses were applied to see if associations varied between women and men and inverse probability weighted regression was used to test if results were sensitive to potential selection bias within the larger UK Biobank cohort.

Overall, results demonstrated consistent associations of higher serum CRP with depressive and anxiety symptoms as well as with probable depression and GAD even after adjusting for age, sex, socioeconomic status, BMI, smoking, alcohol, physical activity, ethnicity, type 2 diabetes and cardiovascular disease. These results followed a linear dose-response pattern and associations of CRP were larger for depression than anxiety outcomes with evidence for disorder-specificity. Although inconsistently, there was some evidence for larger associations in women than in men. MR suggested that CRP had a potential causal risk-decreasing effect on depression and anxiety while IL-6 activity had a risk-increasing effect. This could highlight a potential role of IL-6 trans-signalling rather than IL-6 classic signalling in depression. Future experimental work needs to disentangle IL-6/IL-6R pathways further and clinical trials are needed to test drugs targeting these pathways for depression.

Contributions and reference

The study "Role of Inflammation in Depression and Anxiety: Tests for Disorder Specificity, Linearity and Potential Causality of Association in the UK Biobank" was posted as a preprint on medRxiv in February, 2021. It is currently in revision at EClinicalMedicine. ZY, NK, GMK were responsible for writing of the original draft; ZY, NK, SM, GDS, SB, PBJ, GMK for reviewing and editing the manuscript; ZY, NK, SM, GDS, SB, PBJ, GMK for acquisition, analysis or interpretation of data; GMK, ZY, NK for conceptualisation of the study; ZY, NK for visualisations; ZY, NK for statistical analysis; and GDS, SB, PBJ, GMK for supervision.

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Note

The manuscript presented on the subsequent pages represents the most recent version of the manuscript as of June 2021 and as currently under review in EClinicalMedicine. It is identical in content, but has been formatted to align with the format of this thesis.

Role of Inflammation in Depression and Anxiety: Tests for Disorder Specificity, Linearity and Potential Causality of Association in the UK Biobank

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INTRODUCTION

Innate immune dysfunction represents a putative mechanism for depression and other psychiatric disorders opening up the possibility of new treatment approaches distinct from current monoaminergic drugs.[1,2] In depression, for instance, there is evidence of low-grade systemic inflammation as indexed by elevated concentrations of C-reactive protein (CRP >3mg/L) in 21–34% of patients,[3] along with increased concentrations of interleukin-6 (IL-6) and other inflammatory cytokines in blood and in cerebrospinal fluid (CSF).[4–8] A number of randomised controlled trials (RCTs) are now testing the effects of anti-inflammatory drugs in patients with depression (e.g., Khandaker *et al.*[9], NCT02473289, NCT02362529). However, there are key outstanding questions, particularly regarding specificity and causality of association, that require addressing for a clearer understanding of the potential role of inflammation in illness pathogenesis and to inform future clinical trials.

Depressive disorders overlap with anxiety disorders both genetically and clinically.[10,11] Anxiety symptoms now form part of the diagnostic criteria for major depressive disorder (MDD) as "anxious distress specifier" in the diagnostic and statistical manual of mental disorders 5th edition (DSM-5).[12] Preliminary evidence from case-control studies also indicates that inflammation could be implicated in generalized anxiety disorder (GAD), although findings from studies are mixed and prospective studies indicate that inflammation could increase subsequent to the development of an anxiety disorder.[13,14] Additionally, to our knowledge no studies have tested whether inflammation is a common or specific risk factor for depression and anxiety. This is an important issue as it may help to identify potentially unique or shared mechanisms for psychiatric disorders that commonly co-occur.

Regarding causality, longitudinal studies and meta-analyses have reported evidence for a temporal association between elevated CRP and IL-6 concentrations at baseline and risk of depressive symptoms subsequently,[15–18] but other studies have not fully replicated associations of these markers with subsequent depressive disorders[19,20] and residual confounding still remains a possibility. Mendelian randomization (MR) is an epidemiological approach that uses genetic variants as instruments to untangle the problem of unmeasured confounding as genetic variants are randomly inherited from parents to offspring and fixed at conception.[21] Therefore, if genetically-predicted values of a risk factor are associated with a disease outcome, then it is likely the association between the risk factor and outcome has a causal basis.

Existing MR studies have provided mixed evidence on the association of inflammation with different psychiatric disorders. Hartwig *et al.* reported potential protective effects of elevated CRP for schizophrenia,[22] contrasting with findings from observational studies.[23,24] For depression, one study did not find evidence for a potential causal role of inflammation,[25] while more recent studies reported potential causal roles for increased IL-6 and CRP serum concentrations in depression,[26] for increased IL-6 activity for suicidality specifically,[27] and for increased soluble IL-6R levels for recurrent depressive symptoms.[28] While these findings may indicate disorder-specificity, further research is required to enable definite conclusions regarding causality of association. Furthermore, to our knowledge, MR studies of inflammation and anxiety have thus far only investigated individual anxiety symptoms.[29]

We have used data from up to 144,890 individuals from the UK Biobank study, a large general population-based cohort, to test associations of circulating CRP concentrations with depression and anxiety. As outcomes, we have used symptom scores and categorical probable diagnosis in the total sample and in men and women separately to assess po-

tential sex difference, strength and reproducibility of association. We have examined evidence for dose-response by testing linearity of association. We have examined specificity of association by testing whether the association of CRP with depression and anxiety is stronger for one outcome than the other, or is similar between outcomes. Furthermore, we have carried out MR analysis in the full sample, and in men and women separately, to test whether associations of CRP and IL-6 with depression and anxiety are consistent with potential causal roles for these biomarkers in these conditions.

METHODS

Study population

The UK Biobank is a population-based cohort with a range of phenotyping assessments, biochemical assays and genome-wide genotyping from over 500,000 UK residents aged 40-69 years at baseline, recruited between 2006 and 2010 from 22 assessment centres throughout the UK.[30] Our primary outcomes were depressive and anxiety symptoms that were assessed online as part of a follow-up mental health survey completed by up to 157,115 individuals between July 2016 and July 2017.[31] The current study used available data from the maximum number of UK Biobank participants for each analysis (N up to 144,890). The UK Biobank study was subject to ethics committee approval and participants gave their informed consent prior to participation; see details in Supplementary Methods.

Exposure

Using blood samples collected in the UK Biobank baseline visit between 2006 and 2010 or the first repeat assessment visit between 2012 and 2013, serum high-sensitivity CRP concentrations were measured by immunoturbidimetric assay on a Beckman Coulter AU5800. Minimum detection limit was 0.08 mg/L. CRP values in the entire sample (n=486,424) ranged from 0.08 to 79.96 mg/L; mean=2.60 (SD=4.36) mg/L. The distribution of CRP concentrations for this study (n=146,954) was divided into quintiles or deciles, which were used as categorical variables. We also carried out additional analyses using CRP as a continuous variable (natural log-transformed).

Outcomes

Our primary outcomes were depressive and anxiety symptoms occurring in the last 2 weeks as measured using the Patient Health Questionnaire (PHQ)-9 and the Generalised Anxiety Disorder (GAD)-7 questionnaire, respectively.[32,33] Symptoms were coded as 0-3 depending on self-reported severity. We created sum-scores for each scale, which were

used as primary outcomes. Categorical diagnoses of probable depression and GAD were used as secondary outcomes, which were defined using commonly used cut-off criteria of PHQ-9≥10 and GAD-7≥10. See details in the Supplementary Appendix.

Covariates

As covariates, we included age, sex, body mass index (BMI), smoking, alcohol use, physical activity, ethnicity, Townsend Deprivation Index (TDI), and diabetes and cardiovascular disease; see Supplementary Appendix for details.

Statistical Analyses

Analyses were performed using Stata/SE 16.0 (Stata, College Station, TX). Baseline characteristics of participants were examined across CRP quintiles.

Association of CRP with depression and anxiety, linearity and sex difference

Linear regression was used to estimate the associations between CRP concentrations (quintiles or deciles) and depressive and anxiety symptom scores. For the purpose of interpretation, coefficient estimates were anti-log transformed to odds ratio and 95% confidence interval (CI). We adjusted regression models for age, sex, BMI, smoking, alcohol use, physical activity, ethnicity, TDI, and diabetes and cardiovascular disease.

To investigate the nature of associations with depressive and anxiety symptoms and any dose-response effect in greater detail, CRP concentrations were divided into deciles with deciles 2-10 compared with the lowest decile group (decile 1). Floating absolute risks were estimated, which were then plotted against the median CRP concentrations in each decile. We computed ORs for trend by using quintile number as predictor. We assessed potential quadratic associations by including a quadratic term (CRP-squared). We performed sex-stratified analyses and also tested for interaction between sex and CRP by including interaction terms in regression models. Lastly, we evaluated the influence of selection/collider bias for participation in the optional mental health survey using inverse probability weighted regression of the fully adjusted regression models of depression and anxiety outcomes on CRP;[34,35] see Supplementary Methods for details.

Test for specificity vs commonality of association of CRP between depression and anxiety

We used bivariate probit regression to test for specificity of association of CRP between depression and anxiety using both continuous and categorical outcomes. Probit regression jointly modelled the outcomes of depression and anxiety with CRP, and then tested for equality of regression parameters expressing the effect of CRP on each outcome using the likelihood ratio test. We compared a model that allowed estimates to differ between outcomes with a model where estimates were constrained to be equal for both outcomes. Probit estimates were converted into ORs by multiplying probit parameters by 1.6.[36] In

addition, we adjusted the regression models of depression for anxiety (along with other covariates) and *vice versa* as additional tests for disorder specificity.

Mendelian randomisation approach

Genotyping

We used genotyping data of 342,081 unrelated individuals of White ancestry; see Supplementary Methods for details on genotyping array, central and post-imputation quality control. We used a summary-based approach for MR analyses,[37] so sample sizes differed for estimation of SNP-exposure and SNP-outcome associations. For estimation of SNP-outcome associations, sample sizes varied between 100,739-110,173 per outcome; see Supplementary Table 1 for sample sizes for SNP-exposure associations.

SNP selection

We selected genetic variants in the *CRP* and *IL-6 receptor* (*IL6R*) gene regions previously shown to be associated with CRP or IL-6 concentrations (Supplementary Table 1).[38–41] Genetic instruments differ in strength based on the precision with which they have been estimated in original GWAS studies. As instrument strength informs statistical power for MR analysis, we used genetic instruments from Georgakis *et al.*[38] for primary MR analysis, which have the largest strength (Supplementary Table 1), and report results from other instruments[39–41] as sensitivity analysis.

We extracted SNP-exposure estimates from previous reports to perform 2-sample MR analysis. Based on availability of CRP concentrations in the UK Biobank study, which can be used as downstream readout of IL-6 activity under the classic IL-6 signalling pathway,[38] we also estimated SNP-exposure associations (for 1-sample MR) and SNP-outcome associations, in the full sample and separately for men and women for sex-stratified MR; see details in Supplementary Methods and Supplementary Figure 1.

Mendelian randomisation analyses

We performed MR analysis using inverse-variance weighted (IVW) regression of the genetic associations with the outcome on the genetic associations with the exposure.[37] To evaluate the potential impact of selection/collider bias for participation in the optional mental health survey, we repeated IVW MR analyses with SNP-outcome associations obtained using inverse probability weighted regression.[34] We also evaluated potential horizontal pleiotropy using Cochran's *Q*.[37] See details in Supplementary Appendix.

RESULTS

Baseline Characteristics

In 146,954 participants (43.6% men), mean age at recruitment was 56.5 (SD=7.8) years. Median CRP concentration was 1.15 mg/L (IQR=0.58-2.38 mg/L). Table B1 shows characteristics of study participants by CRP quintiles. Mean depressive symptom scores were 2.76 (SD=3.70, range: 0-27) and mean anxiety symptom scores 2.15 (SD=3.41, range: 0-21); these scores exhibited a moderate-to-large correlation (Pearson's r=0.68). 5.5% of individuals qualified for a probable diagnosis of depression, 4.4% for a probable diagnosis of GAD, and 0.6% for both probable depression and probable GAD.

Table B1. Baseline characteristics of study participants by quintiles of CRP levels in the UK Biobank cohort (n=146,954)

Study characteristics	Q1 (n=34,787)	Q2 (n=32,125)	Q3 (n=29,113)	Q4 (n=26,733)	Q5 (n=24,196)	*P* value
CRP (mg/L) median (range)	0.36 (0.08-0.55)	0.77 (0.56-1.02)	1.33 (1.03-1.75)	2.33 (1.76-3.33)	5.42 (3.34-78.22)	<0.001
Age (years)	54.3 (7.8)	55.82 (7.7)	56.5 (7.6)	56.9 (7.6)	56.6 (7.7)	<0.001
Women (%)	20262 (58.3)	17255 (53.7)	15588 (53.5)	14867 (55.6)	14931 (61.7)	<0.001
White ethnicity (%)	33601 (96.6)	31166 (97.0)	28228 (97.0)	25907 (96.9)	23399 (96.7)	<0.001
TDI, median (SD)	-1.7 (2.8)	-1.8 (2.8)	-1.8 (2.8)	-1.7 (2.8)	-1.5 (2.9)	<0.001
BMI (kg/m^2)	24.1 (3.1)	25.8 (3.4)	27.0 (3.9)	28.2 (4.3)	30.1 (5.8)	<0.001
Smoking status (%)						
Never	21603 (62.1)	18927 (58.9)	16509 (56.7)	14722 (55.1)	12555 (51.9)	
Current	1965 (5.7)	1981 (6.2)	2057 (7.1)	2162 (8.1)	2418 (10.0)	
Ex-smokers	11157 (32.1)	11138 (34.7)	10484 (36.0)	9783 (36.6)	9163 (37.9)	<0.001
Alcohol status (%)						
Never/Ex	1743 (5.0)	1581 (4.9)	1578 (5.4)	1633 (6.1)	1659 (6.9)	
Occasional (≤ 3 times per week)	14376 (41.3)	13856 (43.2)	13052 (44.8)	12719 (47.6)	12184 (50.4)	
Regular (> 3 times per week)	18657 (53.7)	16677 (51.9)	14475 (49.7)	12369 (46.3)	10342 (42.8)	<0.001
Physical activity (%)						
Inactivity	27490 (90.0)	24961 (80.1)	22180 (79.1)	19756 (77.7)	16816 (74.9)	
Moderately inactive	1350 (4.0)	1548 (5.0)	1633 (5.8)	1742 (6.9)	1969 (8.8)	
Moderately active	4342 (12.8)	3881 (12.5)	3443 (12.3)	3206 (12.6)	2967 (13.2)	
Active	779 (2.3)	778 (2.5)	780 (2.8)	722 (2.8)	711 (3.2)	<0.001
Diabetes (%)	780 (2.2)	881 (2.7)	983 (3.4)	1022 (3.8)	1210 (5.0)	<0.001
Cardiovascular disease (%)	1029 (3.0)	1093 (3.4)	1076 (3.7)	1035 (3.9)	973 (4.0)	<0.001

Note: Differences were estimated using mean and SD for continuous variables, with p-values from ANOVA test, or using number and percent for categorical variables, with $\chi 2$ test.

Association of CRP Concentration with Depressive and Anxiety Symptom Scores

Results for associations of CRP with depressive and anxiety symptoms are presented in Figure B1 across different CRP deciles in the total sample, and for women and men separately in Supplementary Figures 2 and 3. Overall, CRP was associated with depressive and anxiety symptoms after adjusting for all potential confound factors, but adjustment for BMI attenuated these associations to some extent (Supplementary Tables 2 & 3).

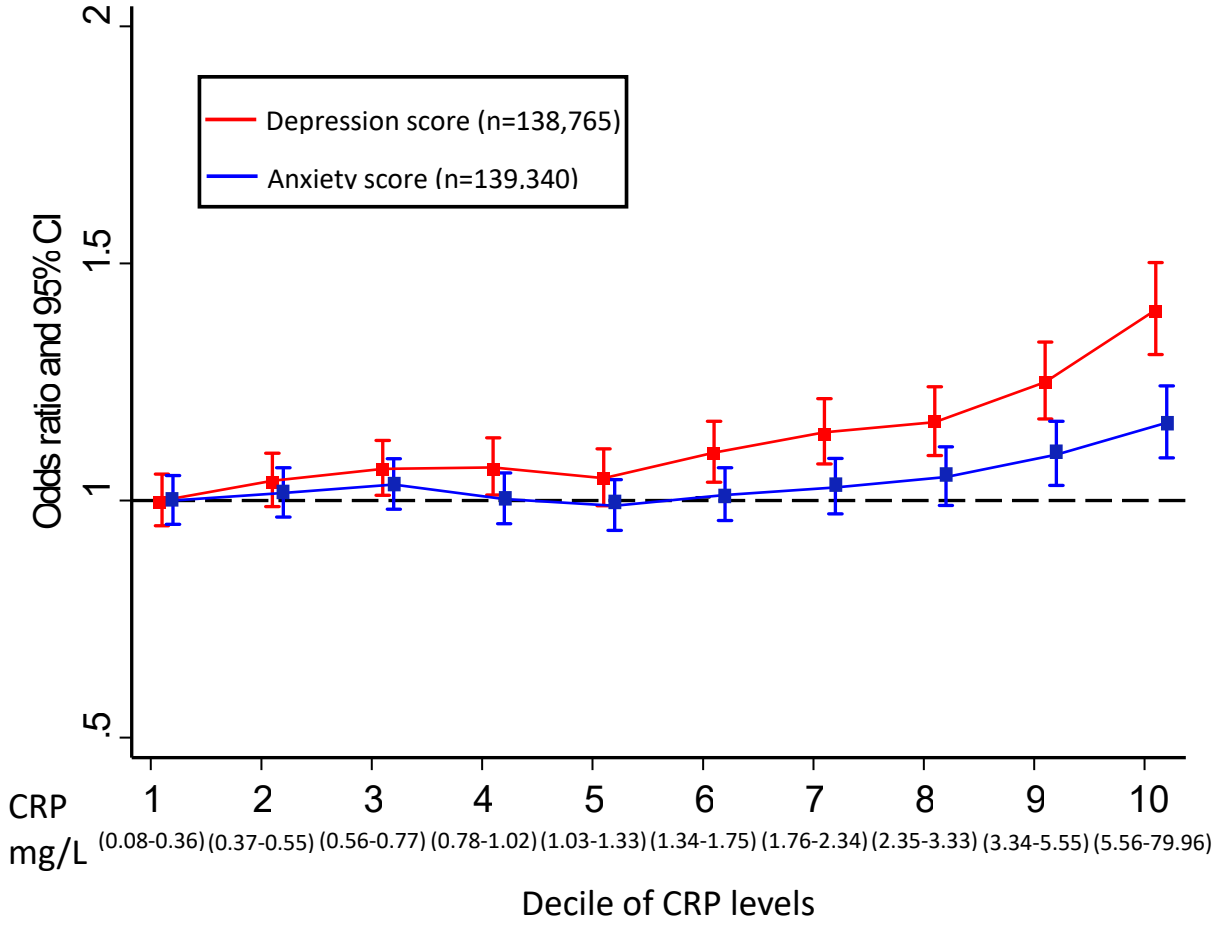

Figure B1. Odds ratios for higher depressive and anxiety symptom scores per decile of CRP levels in the UK Biobank cohort. CRP: C-reactive protein; Confidence intervals (CIs) were calculated using a floating absolute risk technique; Odds ratios were adjusted for age, sex, BMI, smoking status, alcohol intake, physical activity, TDI, ethnic group, diabetes and cardiovascular disease; red: depression score; blue: anxiety score

Using CRP as a continuous variable, the adjusted OR for higher depressive symptom score per-unit increase in log CRP was 1.09 (95% CI, 1.06-1.11). Using CRP as a categorical variable, the adjusted OR for higher depressive symptom score for participants in the top, compared with bottom, quintile of CRP was 1.29 (95% CI, 1.21-1.38). Inverse probability weighted regression analyses of depressive symptoms did not suggest that results were

affected by collider bias, as the adjusted OR=1.31 (95% CI, 1.22-1.41) for participants in the top, compared with bottom, quintile of CRP was similar.

Using CRP as a continuous variable, the adjusted OR for higher anxiety symptom score per-unit increase in log CRP was 1.03 (95% CI, 1.02-1.05). Using CRP as a categorical variable, the adjusted OR for higher anxiety symptom score for participants in the top, compared with bottom, quintile of CRP was 1.12 (95% CI, 1.05-1.19). Again, evidence did not suggest results were affected by collider bias with similar OR of 1.12 (95% CI, 1.05-1.20) in sensitivity analyses.

Association of CRP Concentration with Probable Diagnoses of Depression and GAD

CRP was associated with probable diagnosis of depression (Table B2). Using CRP as a continuous variable, the adjusted OR for depression per-unit increase in log CRP was 1.09 (95% CI, 1.06-1.11). Using CRP as a categorical variable, the adjusted OR for depression for participants in the top, compared with bottom, quintile of CRP was 1.29 (95% CI, 1.18-1.40). Evidence did not suggest results were affected by collider bias with similar OR of 1.29 (95% CI, 1.18-1.41) in sensitivity analyses.

CRP was associated with probable diagnosis of GAD (Table B3). Using CRP as a continuous variable, the adjusted OR for GAD per-unit increase in log CRP was 1.05 (95% CI, 1.02-1.08). Using CRP as a categorical variable, the adjusted OR for GAD for participants in the top, compared with bottom, quintile of CRP was 1.15 (95% CI, 1.05-1.26). Again, evidence did not support collider bias as likely explanation with similar OR of 1.13 (95% CI, 1.02-1.24) in sensitivity analyses.

Table B2. Association of C-reactive protein levels with probable diagnosis of depression in the UK Biobank cohort

	log CRP as continuous variable	CRP Q1 (n=34,372)	CRP Q2 (n=31,704)	CRP Q3 (n=28,714)	CRP Q4 (n=26,350)	CRP Q5 (n=23,750)	Per-Q effect	*P*-value for trend
All participants (cases = 8,888; controls = 145,468)								
Model 1 (n=144890)	1.27 (1.24-1.29)	1 [reference]	1.11 (1.03-1.19)	1.19 (1.10-1.28)	1.44 (1.34-1.54)	2.05 (1.91-2.20)	1.19 (1.17-1.21)	<0.001
Model 2 (n=144600)	1.12 (1.09-1.15)	1 [reference]	1.08 (1.00-1.16)	1.10 (1.02-1.18)	1.22 (1.13-1.31)	1.41 (1.31-1.53)	1.09 (1.07-1.10)	<0.001
Model 3 (n=138766)	1.09 (1.06-1.11)	1 [reference]	1.07 (0.99-1.15)	1.08 (1.00-1.17)	1.16 (1.07-1.26)	1.28 (1.18-1.39)	1.06 (1.04-1.08)	<0.001
Model 4 (n=138765)	1.09 (1.06-1.11)	1 [reference]	1.07 (0.99-1.16)	1.08 (1.00-1.17)	1.16 (1.07-1.26)	1.29 (1.18-1.40)	1.06 (1.04-1.08)	<0.001
Women (cases = 5,641; controls = 81,562)								
Model 1 (n=81610)	1.28 (1.25-1.32)	1 [reference]	1.06 (0.96-1.16)	1.22 (1.12-1.34)	1.40 (1.28-1.53)	2.11 (1.94-2.29)	1.20 (1.18-1.23)	<0.001
Model 2 (n=81454)	1.12 (1.08-1.15)	1 [reference]	1.03 (0.94-1.13)	1.13 (1.03-1.24)	1.18 (1.07-1.30)	1.41 (1.27-1.55)	1.09 (1.06-1.11)	<0.001
Model 3 (n=77818)	1.10 (1.06-1.14)	1 [reference]	1.02 (0.93-1.13)	1.13 (1.02-1.25)	1.17 (1.06-1.30)	1.33 (1.20-1.48)	1.07 (1.05-1.10)	<0.001
Model 4 (n=77818)	1.10 (1.06-1.13)	1 [reference]	1.02 (0.93-1.13)	1.13 (1.02-1.25)	1.17 (1.06-1.30)	1.33 (1.20-1.48)	1.07 (1.05-1.10)	<0.001
Men (cases = 3,247; controls = 63,906)								
Model 1 (n=63280)	1.22 (1.18-1.27)	1 [reference]	1.23 (1.09-1.38)	1.17 (1.04-1.32)	1.53 (1.36-1.72)	1.87 (1.66-2.11)	1.16 (1.13-1.19)	<0.001
Model 2 (n=63146)	1.13(1.08-1.17)	1 [reference]	1.16 (1.03-1.30)	1.04 (0.92-1.18)	1.27 (1.12-1.43)	1.44 (1.27-1.64)	1.08 (1.05-1.12)	<0.001
Model 3 (n=60948)	1.07 (1.02-1.11)	1 [reference]	1.12 (0.99-1.27)	1.00 (0.88-1.14)	1.14 (1.00-1.29)	1.21 (1.06-1.39)	1.04 (1.01-1.07)	0.02
Model 4 (n=60947)	1.07 (1.03-1.12)	1 [reference]	1.13 (1.00-1.28)	1.01 (0.89-1.15)	1.15 (1.01-1.31)	1.23 (1.07-1.41)	1.04 (1.01-1.07)	0.01

Note: Data show OR and 95% CIs unless otherwise indicated. P for trend is from regression models with quintiles. Model 1, unadjusted; model 2, adjusted for age, sex, and BMI (body mass index); model 3, model 2 additionally adjusted for smoking, alcohol, physical activity, ethnicity, and TDI (Townsend deprivation index at recruitment); model 4, model 3 additionally adjusted for diabetes and cardiovascular disease; *: CRP concentration was log transformed; Median CRP level was 1.15 mg/L (range 0.08-78.22 mg/L)

Table B3. Association of C-reactive protein levels with probable GAD diagnosis in the UK Biobank cohort

	log CRP as continuous variable	CRP Q1 (n=34,499)	CRP Q2 (n=31,809)	CRP Q3 (n=28,829)	CRP Q4 (n=26,451)	CRP Q5 (n=23,950)	Per-Q effect	*P* for trend
All participants (cases = 6,395; controls = 139,143)								
Model 1 (n=145,538)	1.11 (1.08-1.14)	1 [reference]	0.95 (0.88-1.03)	0.95 (0.88-1.03)	1.05 (0.97-1.13)	1.38 (1.28-1.49)	1.08 (1.06-1.10)	<0.001
Model 2 (n=145,239)	1.07 (1.04-1.10)	1 [reference]	0.99 (0.91-1.07)	0.99 (0.91-1.07)	1.05 (0.97-1.14)	1.24 (1.14-1.36)	1.05 (1.03-1.07)	<0.001
Model 3 (n=139,341)	1.05 (1.02-1.08)	1 [reference]	0.97 (0.90-1.06)	0.99 (0.91-1.07)	1.02 (0.94-1.12)	1.15 (1.05-1.26)	1.03 (1.01-1.05)	0.004
Model 4 (n=139,340)	1.05 (1.02-1.08)	1 [reference]	0.98 (0.90-1.06)	0.98 (0.90-1.07)	1.02 (0.94-1.11)	1.15 (1.05-1.26)	1.03 (1.01-1.05)	0.005
Women (cases = 4,247; controls = 77,717)								
Model 1 (n=81,964)	1.10 (1.07-1.13)	1 [reference]	0.97 (0.88-1.07)	0.95 (0.86-1.05)	1.03 (0.93-1.13)	1.38 (1.26-1.51)	1.07 (1.05-1.10)	<0.001
Model 2 (n=81,799)	1.08 (1.04-1.11)	1 [reference]	1.00 (0.91-1.10)	0.98 (0.88-1.08)	1.05 (0.94-1.16)	1.29 (1.16-1.43)	1.05 (1.03-1.08)	<0.001
Model 3 (n=78,110)	1.07 (1.03-1.10)	1 [reference]	0.98 (0.89-1.09)	0.99 (0.90-1.10)	1.04 (0.94-1.16)	1.23 (1.10-1.38)	1.05 (1.02-1.07)	0.001
Model 4 (n=78,110)	1.06 (1.03-1.10)	1 [reference]	0.99 (0.89-1.09)	0.99 (0.89-1.10)	1.04 (0.93-1.16)	1.23 (1.10-1.37)	1.05 (1.02-1.07)	0.001
Men (cases = 2,148; controls = 61,426)								
Model 1 (n=63,574)	1.10 (1.06-1.16)	1 [reference]	0.97 (0.85-1.11)	1.02 (0.89-1.17)	1.12 (0.98-1.28)	1.33 (1.16-1.53)	1.07 (1.04-1.11)	<0.001
Model 2 (n=63,440)	1.07 (1.02-1.12)	1 [reference]	0.97 (0.85-1.11)	1.02 (0.89-1.17)	1.12 (0.98-1.28)	1.33 (1.16-1.53)	1.04 (1.01-1.08)	0.018
Model 3 (n=61,231)	1.02 (0.98-1.07)	1 [reference]	0.94 (0.82-1.08)	0.95 (0.83-1.10)	0.97 (0.84-1.13)	1.02 (0.87-1.20)	1.01 (0.97-1.04)	0.74
Model 4 (n=61,230)	1.02 (0.98-1.07)	1 [reference]	0.95 (0.82-1.08)	0.95 (0.83-1.10)	0.97 (0.84-1.13)	1.02 (0.87-1.20)	1.01 (0.97-1.04)	0.74

Note: Data show ORs and 95% CIs unless otherwise indicated. P for trend is from regression models with quintiles. Model 1, unadjusted; model 2, adjusted for age, sex, and BMI (body mass index); model 3, model 2 additionally adjusted for smoking, alcohol, physical activity, ethnicity, and TDI (Townsend deprivation index at recruitment); model 4, model 3 additionally adjusted for diabetes and cardiovascular disease; *: CRP concentration was log transformed; Median CRP level was 1.33 mg/L (range 0.08-79.96 mg/L).

Test for specificity vs commonality of association of CRP with depression and anxiety

In bi-variate probit regression analysis, we found evidence for a stronger association of CRP with depressive symptoms (OR=1.014; 95% CI, 1.011-1.017) than anxiety symptoms (OR=1.004; 95% CI, 1.002-1.007). Results for probit regression using probable diagnoses of depression and GAD as outcomes were similar (see Supplementary Results).

In regression analyses, evidence for association of CRP with depression symptoms remained after adjusting for anxiety symptoms (OR=1.06; 95% CI, 1.05-1.08), but the association of CRP with anxiety symptoms switched its valence after adjusting for depressive symptoms (OR=0.98; 95% CI, 0.97-0.99).

Linearity of association

Evidence was compatible with linear associations of CRP with both depression and anxiety across all analyses using symptom scores and probable diagnoses as outcomes (*P*-value for all quadratic terms >0.05).

Examination of potential sex difference

In sex-stratified analyses, point estimates were larger for women than men for both depression and anxiety symptom outcomes (Supplementary Tables 2-3, Supplementary Figures 2-3). However, evidence for an interaction between CRP and sex was present only for depressive symptoms (adjusted OR_{women}=1.35; 95%CI, 1.23-1.48; adjusted OR_{men}=1.21; 95%CI, 1.10-1.33; *P*-value for interaction term=0.032). For categorical outcomes, point estimates were larger for women for probable GAD (Tables 2-3), but evidence did not support interaction for either outcomes (all *P*>0.2).

Results for Mendelian randomization analyses

Genetically-predicted concentration/activity of IL-6 and CRP were associated with both depression and anxiety. However, these associations differed with regards to direction of association (i.e., increased vs decreased risk), particular outcome definition, and sex. Table B4 shows results for IVW MR analyses based on Georgakis *et al.*[38] genetic instruments for CRP and IL-6.

Table B4. IVW Mendelian randomisation analysis of association of IL-6 and CRP with depression and anxiety

	Depression Symptom Score		Probable depression		Anxiety Symptom Score		Probable GAD	
Model	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**	**OR (95% CI)**	***P*-value**
CRP								
2-Sample MR	0.88 (0.80-0.98)	0.020	0.95 (0.85-1.07)	0.424	0.87 (0.80-0.95)	0.003	0.82 (0.72-0.94)	0.004
1-Sample MR	0.89 (0.79-1.00)	0.055	1.01 (0.88-1.14)	0.939	0.88 (0.79-0.97)	0.008	0.84 (0.73-0.98)	0.027
Women	0.98 (0.85-1.12)	0.754	1.12 (0.96-1.30)	0.152	0.86 (0.76-0.98)	0.023	0.85 (0.72-1.01)	0.059
Men	0.78 (0.63-0.96)	0.018	0.84 (0.66-1.06)	0.138	0.91 (0.78-1.05)	0.192	0.83 (0.62-1.11)	0.209
IL-6								
2-Sample MR	1.34 (1.05-1.72)	0.019	1.15 (0.86-1.54)	0.340	1.13 (0.91-1.41)	0.269	1.24 (0.89-1.73)	0.194
1-Sample MR	1.32 (1.03-1.67)	0.025	1.18 (0.89-1.56)	0.246	1.11 (0.90-1.37)	0.313	1.18 (0.86-1.62)	0.297
Women	1.42 (1.01-1.97)	0.041	1.46 (1.00-2.13)	0.048	1.15 (0.85-1.56)	0.362	1.51 (1.01-2.25)	0.044
Men	1.24 (0.88-1.74)	0.218	0.86 (0.54-1.37)	0.516	1.08 (0.79-1.47)	0.636	0.79 (0.47-1.33)	0.385

Note: Estimates for men and women are based on sex-stratified 1-sample MR analyses.

For CRP, per-unit increase in genetically-predicted concentrations of log-transformed CRP was associated with lower risk for depressive symptoms (1-sample MR: OR=0.89; 95% CI, 0.79-1.00; 2-sample MR: OR=0.88; 95% CI, 0.80-0.98), and lower risk for anxiety symptoms (1-sample MR: OR=0.88; 95% CI, 0.79-0.97; 2-sample MR: OR=0.87; 95% CI, 0.80-0.95). Using the categorical outcomes, MR analyses also showed that increased genetically-predicted CRP was associated with lower risk for probable GAD, but point estimates for probable depression were close to one (Table B4). In sex-stratified MR analyses, higher genetically predicted CRP concentrations were associated with relatively lower risk for depressive symptoms in men, and with relatively lower risk for anxiety symptoms in women.

For IL-6, per-unit increase in higher genetically-predicted IL-6 activity was associated with increased risk for depressive symptoms (1-sample MR: OR=1.32, 95% CI 1.03-1.67; 2-sample MR: OR=1.34, 95% CI 1.05-1.72), but not with probable depression or either anxiety outcome. In sex-stratified MR analyses, we found evidence that higher genetically-predicted IL-6 activity was associated with increased risk for depressive symptoms, probable depression, and probable GAD in women only.

MR analyses using alternative genetic instruments were directionally consistent with these results, albeit with larger confidence intervals possibly due to the lower statistical power for these instruments (Supplementary Table 4). Results for sensitivity analyses evaluating the impact of selection/collider bias were similar to main IVW analyses (Supplementary Table 5).

Evidence did not suggest directional horizontal pleiotropy was a likely explanation for any of the IVW MR results as assessed using Cochran's Q (Supplementary Table 6).

DISCUSSION

Based on data from the UK Biobank cohort, a large general population cohort, we report that circulating CRP concentrations are associated with depressive and anxiety symptoms and with probable diagnoses of depression and GAD in a linear, dose-response fashion. At the same time, we show evidence for disorder-specificity suggesting that CRP is more strongly associated with depression compared to anxiety. We also found some evidence for sex-specificity. CRP was more strongly associated with depression in women than in men. Using MR analyses, we provide evidence that higher IL-6 activity could represent a potential causal factor increasing depression, while genetically predicted higher CRP concentrations appeared to potentially be protective for depression and anxiety, which contrasts findings for serum CRP.

Associations of inflammation with depression and anxiety

Although inflammation was associated with both depression and anxiety, we report stronger associations for depression outcomes indicating disorder-specificity. This aligns with meta-analyses of case-control studies showing higher concentrations of CRP and other inflammatory markers in depression,[3,4,6–8] while there are relatively fewer studies suggesting this for anxiety.[13] Cohort studies of affective symptoms also suggest that circulating IL-6 and CRP concentrations are predominantly associated with depressive rather than anxiety symptoms.[29] Together, current evidence is consistent with the idea that systemic inflammation may be particularly relevant for depression rather than anxiety disorders.

Our results also provide some evidence for sex-specificity. Associations of serum CRP concentrations with depression and anxiety were mostly stronger in women than men. Results for sex-stratified MR analyses suggested that higher IL-6 could be a risk factor for depressive symptoms specifically for women while higher CRP could be protective for depressive symptoms specifically for men and for anxiety symptoms specifically for women. It is important to note, however, that confidence intervals of sex-stratified MR estimates overlapped between sexes emphasising the tentative nature of these results. Existing evidence on potential sex-difference for associations between inflammatory makers and depression has also been mixed. A previous meta-analysis reported no sex-specificity of the association between CRP and depression.[3] In contrast, two recent studies reported that IL-6 was associated with depressive symptom chronicity and treatment response specifically in women.[20,42] Atypical depression, which is characterised by immuno-metabolic dysregulation, has also been reported to be more common in women.[43] Hitherto most studies have considered sex as a covariate. Further research is needed to replicate our findings regarding potential sex-specificity.

Our findings lend support to RCTs testing immunotherapies targeting the IL-6/IL-6R pathway for patients with depression. Anti-inflammatory treatments have been shown to exhibit antidepressant activity in chronic inflammatory illnesses.[44–46] In depression, initial results suggest that these drugs may be useful for patients with evidence of inflammation and inflammation-related risk factors.[47–49] This hypothesis is now being investigated in ongoing RCTs that are selecting patients based on evidence of inflammation and inflammation-related phenotypes.[9,50] The present study further highlights characteristics associated with inflammation, e.g., female sex, to inform stratified patient selection in future clinical trials.

Potential interpretations for divergent effects of CRP and IL-6

Using genetic variants in the *IL6R* and *CRP* gene loci, we have found that higher genetically predicted IL-6 activity was associated with increased risk of depression, but higher genetically predicted CRP levels were associated with decreased risk of depression. These findings are intriguing because IL-6 signalling is a key driver of CRP response,[51,52] and so we would expect both to affect depression risk in a comparable way. One potential explanation could be that IL-6 classic and trans-signalling have divergent effects on depression risk. We have illustrated this hypothesis in Figure B2, which describes IL-6 signalling pathways and a Directed Acyclic Graph of these pathways incorporating our MR results.

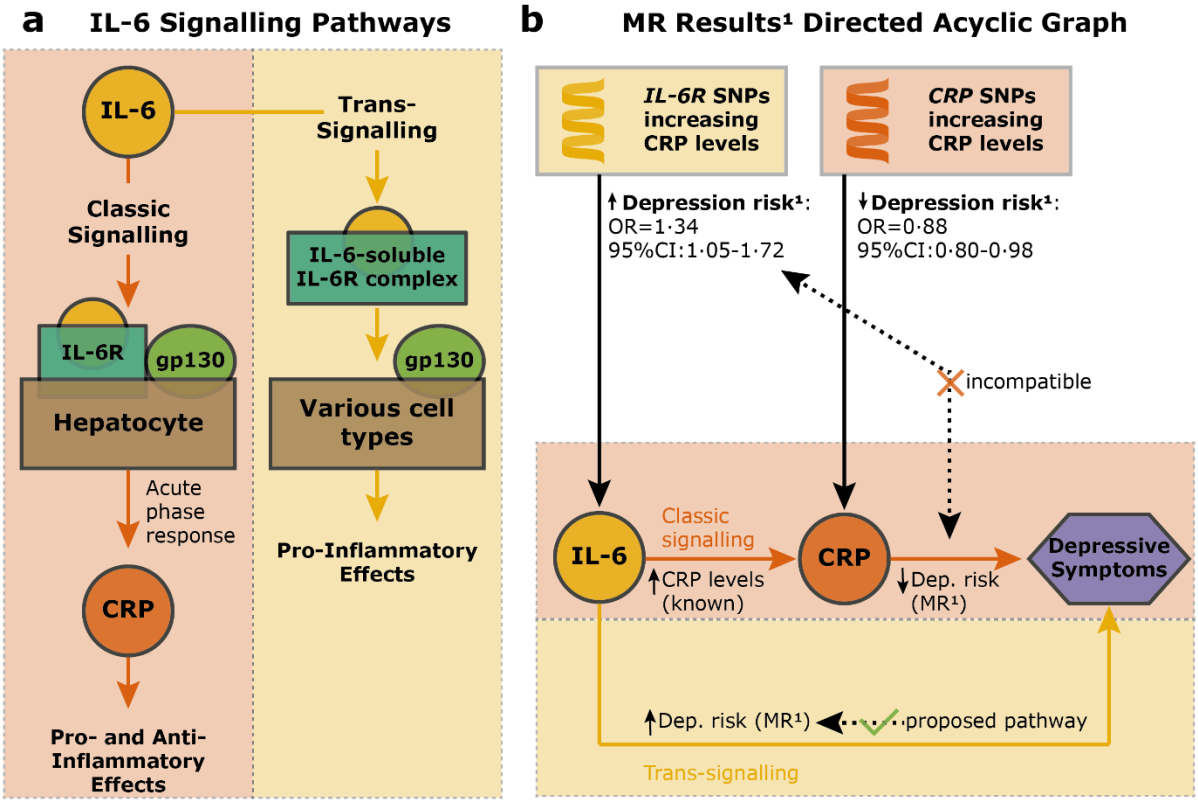

Figure B2. Potential divergent effects of specific IL-6 signalling pathways on depression risk. Figure B2a shows IL-6 classic and trans-signalling pathways; see review by Hunter and Jones [51]. Figure B2b displays our working hypothesis arising from MR results that IL-6 trans-signalling confers increased risk for depression. [1]MR estimates are based on 2-sample MR analysis using Georgakis *et al.* [38] genetic instruments and continuous depressive symptoms as outcome (cf. Table B4). Abbreviations: gp130=glycoprotein 130; Dep.=depression; CRP=C-reactive protein; IL-6=interleukin-6.

In brief, IL-6 classic signalling occurs via its action on membrane-bound IL-6 receptors (IL-6Rs) expressed by limited cell types. IL-6 also binds with circulating soluble IL-6R (sIL-6R) to form an IL-6-sIL-6R complex, which then activates IL-6 signalling by binding

with the ubiquitous glycoprotein 130 on other cells that naturally lack IL-6Rs. This is called IL-6 trans-signalling, which is thought to underlie pro-inflammatory effects of IL-6 in chronic inflammatory diseases.[51]

Mechanistically, the observed increased depression risk conferred by *IL6R* SNPs that increase CRP levels[38] could happen as a result of either increased IL-6 classic or trans-signalling. Our results indicate that it may be due to increased trans-signalling, because we also see that SNPs in the *CRP* gene that increase CRP levels[38] are protective for depression. It is well-known that CRP is mainly produced by hepatocytes as a result of increased IL-6 classic signalling.[51] Taken together, these findings also align with a recent MR study on the effects of genetically predicted sIL-6R, sgp130 (an inhibitor of IL-6 trans-signalling[51]), and CRP on recurrent depression, which suggested that increased IL-6 trans-signalling or decreased IL-6 classic signalling could be responsible for a risk-increase in recurrent depressive symptoms.[28]

While the MR approach can provide evidence supporting causality, as we do here for IL-6 and depression, disentangling the issue of IL-6 classic vs trans-signalling is beyond the scope of population genomics approaches as full effects of genetic variants used are unknown. The field now requires experimental studies of IL-6 modulation in humans and animals to further examine causality, pathogenic mechanisms, and therapeutic potential of anti-IL-6 and other immunotherapies for depression. Findings from these studies may help to devise more targeted IL-6 pathway-specific interventions.

Strengths and Limitations

Strengths of the work include use of a large population-based sample, a range of affective symptoms, and complementary analysis using protein levels and genetic variants. We assessed reproducibility and strength of association using different outcomes and sex-stratified analysis, evidence of linearity and potential causality of associations. Limitations of the work include focus on self-reported symptom score/probable diagnosis. Self-report measures of depression can capture different characteristics than observer-rated measures, so findings need to be replicated using the observer-rated modality.[53] Depression is also a phenotypically heterogeneous syndrome and previous studies have reported that inflammation may be associated with specific symptoms, such as fatigue, changes in appetite and sleep, and suicidality.[27,29,43] Aetiology of depressive symptoms could also vary across the lifespan, so findings from UK Biobank participants (mean age of 57 years) need to be replicated in other age groups. Second, although there was little evidence that associations of CRP with depression and anxiety could be due to selection/collider bias into the optional UK Biobank Mental Health Survey, selection/collider bias for participation in the UK Biobank cohort itself would likely be larger and remains

a possible explanation for our findings that we could not explore. This is particularly relevant as the UK Biobank study includes individuals who are among others older, more likely to be women, healthier and of higher socioeconomic status compared to the general UK population.[54] Third, MR findings were based on a subgroup of individuals of European ancestry, which is a common issue in genetic studies, warranting replication in other ethnic groups. Finally, IL-6 was not measured in the UK Biobank cohort, so we were unable to assess associations of serum IL-6 concentrations with depression and anxiety.

Conclusions

We report evidence for associations of higher serum CRP concentrations with depressive and anxiety symptoms, which are stronger for depressive than for anxiety symptoms and, although less consistently, for women than for men. Findings from MR analyses are consistent with a causal role of altered activity of the IL-6/IL-6R pathway in depressive symptoms, suggesting that this pathway could be a promising, new therapeutic target for depression. Due to uncertainties regarding the full functional effects of genetic variants used as MR instruments, the field now requires human and animal experimental studies to elucidate mechanisms for divergent effects for CRP and IL-6 on illness risk. This may help to devise more targeted interventions.

CONFLICTS OF INTEREST

The authors declare no conflict of interest with regards to the content of this study.

ROLE OF THE FUNDING SOURCE

The funding sources had no role in study design; collection, analysis, and interpretation of data; writing of the report; and the decision to submit the paper for publication.

DATA SHARING

UK Biobank data can be accessed through formal application to the cohort. GWAS summary data used as part of this report are freely available online or can be requested for CRP from the CHARGE inflammation working group. Genetic instrument estimates and scripts for MR processing and analysis are made available online for full reproducibility under https://osf.io/apme9/.

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