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Depression and interleukin-6 signaling: A Mendelian Randomization Study

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Abstract

Background: A large body of research has reported associations between depression and elevated interleukin-6 (IL-6), a cytokine with several roles including pro-inflammatory signaling. The nature and directionality of this relationship are not yet clear. In this study we use Mendelian Randomization to examine the possibility of a causal relationship between IL-6 and depressive symptoms, and to explore multiple signaling pathways that could serve as mechanisms for this relationship.

Methods: This study uses a two-sample Mendelian Randomization design. Data come from the UK Biobank (n=89,119) and published summary statistics from six existing GWAS analyses. The primary analysis focuses on the soluble interleukin-6 receptor (sIL-6R), which is involved in multiple signaling pathways. Exploratory analyses use C-reactive protein (CRP) and soluble glycoprotein 130 (sgp130) to further examine potential underlying mechanisms.

Results: Results are consistent with a causal effect of sIL-6R on depression (PCA-IVW Odds Ratio: 1.023 (95% Confidence Interval: 1.006–1.039), p=0.006). Exploratory analyses demonstrate that the relationship could be consistent with either decreased classical signaling or increased trans signaling as the underlying mechanism.

Discussion: These results strengthen the body evidence implicating IL-6 signaling in depression. When compared with existing observational and animal findings, the direction of these results suggests involvement of IL-6 trans signaling. Further study is needed to examine whether *IL6R* genetic variants might influence IL-6 trans signaling in the brain, as well as to explore other potential pathways linking depression and inflammation.

Keywords

Depression; inflammation; Mendelian Randomization; interleukin-6; soluble interleukin-6 receptor; sIL-6R

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1 Introduction

A large body of literature reports that depression is associated with elevated levels of inflammatory biomarkers.[1–6] The reasons for this association are not yet fully understood, and the association could operate by way of several different neurobiological pathways. The cytokine interleukin-6 (IL-6) has a widely-replicated association with depressive symptoms, and may represent a plausible biological pathway through which inflammation could contribute to depressive symptoms.[4–8]

IL-6 is involved in brain signaling related to “sickness behavior”, an adaptive response to illness or injury that leads to behavioral changes such as reduced appetite and decreased activity.[3,9,10] IL-6 signaling is also associated with reduced neurogenesis in the hippocampus,[11,12] parallelling the reduced hippocampal volumes observed in individuals with depression.[13,14] Experimental studies in humans and animals support the possibility of a causal relationship between IL-6 and depressive symptoms. A small human study (n=16) showed that injection with IL-6 produced short-term depression-like alterations in mood.[15] In mice exposed to experimental stressors, IL-6 receptor blockade[16] and IL-6 knockout mutations[17] have been found to reduce development of depression-like behaviors. Similarly, in rats, blocking IL-6 receptors reduced sickness behavior after injection with lipopolysaccharide, an inflammation-provoking agent.[10]

IL-6 has two major signaling pathways, which have different mechanisms through which they could interact with the brain and influence risk of depression. In classical IL-6 signaling, IL-6 binds to a membrane-bound receptor (mIL-6R), which is found only on certain types of cells (such as liver cells and immune cells).[18–20] In trans IL-6 signaling, IL-6 binds to a soluble IL-6 receptor (sIL-6R) in circulation, and the IL-6/sIL-6R complex is then capable of interaction with cells, including those that lack membrane IL-6 receptors.[18,21] Much of the interaction between IL-6 and the brain occurs via the trans pathway,[22,23] and animal models have confirmed an important role for the trans pathway in neuroinflammation[24] and sickness behavior,[25] suggesting that IL-6 trans signaling is plausible as the relevant pathway in depression.[22] Additionally, animal studies have shown substantial reductions in inflammation-induced sickness behavior when using inhibitors specific to the trans pathway.[25,26] However, there are also mechanisms through which classical IL-6 signaling could influence the brain, including activity of microglia (which have a membrane-bound receptor),[27] and the effect of classical IL-6 signaling on immunoregulation and other cytokines,[20,28] as shown in Figure 1. Identifying the specific pathway involved is of particular interest due to ongoing efforts to develop depression treatments which target IL-6 signaling.

Although it is plausible that IL-6 signaling plays a causal role in depression, alternative explanations for the association are also possible. These explanations include reverse causality, in which depression or depression-related health behaviors lead to increases in IL-6 signaling,[29] or confounding by factors such as socioeconomic status[30] that are associated with both depression and inflammatory signaling. The Mendelian Randomization study design is capable of distinguishing between such competing explanations. In Mendelian Randomization, a genetic variant with a known biological effect is used as

an instrumental variable to assess the causal relationship between levels of an exposure or biomarker influenced by the genetic instrumental variable and an outcome of interest, as illustrated in Figure 2.[31] Given certain assumptions such as the absence of population genetic stratification, genotype at the selected locus will be randomly distributed throughout the population, creating randomized “exposure groups” similar to those used in clinical trials.[32]

While the Mendelian Randomization design has major strengths as a means of testing causal hypotheses, the complexity of the IL-6 signaling system makes it a difficult target for Mendelian Randomization. Relatively few significant GWAS findings are available for levels of the IL-6 protein itself, and the identified SNPs do not meet the requirements for Mendelian Randomization.[33] Although more promising genetic instruments are available for the IL-6 receptor, the *IL6R* gene encodes both the soluble and membrane-bound forms of the IL-6 receptor, and variants in this gene have the potential to influence both classical and trans signaling. For example, the SNP rs2228145 is a missense variant in a proteolytic cleavage site involved in releasing sIL-6R in its soluble form.[34] The minor allele is associated with higher levels of sIL-6R, which results in reduction of IL-6 classical signaling (attributable to lower levels of mIL-6R or to buffering of IL-6 in circulation by excess sIL-6R and sgp130),[34,35] and an increase in the levels and half-life of IL-6 itself.[36,37] The effects of this variant on trans signaling are not as well understood. Although it increases both IL-6 and sIL-6R, it does not change levels of sgp130. The higher abundance of sgp130 relative to IL-6 and sIL-6R is predicted to prevent these changes from increasing trans signaling,[35] although differing concentrations found in particular tissues[35] or under certain physiological conditions[38] may overcome this inhibition. Additionally, a recent study has suggested that sgp130 may have less than the expected ability to inhibit trans signaling at typical physiologic concentrations.[39]

Two recent publications have applied Mendelian Randomization using variants in the *IL6R* gene to examine the relationship between IL-6 signaling and depression. A study by Khandaker et al. (2019) used variants in the *IL6R* gene region as instrumental variables for interleukin-6 signaling, and found evidence consistent with a causal effect on depression.[40] However, this study also noted that variants in the *IL6R* gene region had opposing effects (alleles that increased IL-6 levels also decreased classical signaling), and stated that further investigation was necessary to fully understand these findings. Another recent Mendelian Randomization used inversed values for SNP coefficients with sIL-6R to reflect its dampening effect on classical IL-6 signaling.[41] This analysis reported an association between IL-6 signaling and suicidality, but found no overall association between IL-6 signaling and depression. These studies offer some evidence of a causal relationship, however further study is needed to confirm the relationship between IL-6 signaling and depression, and to determine which of the IL-6 signaling pathways is involved.

In this study we use Mendelian Randomization to test the hypothesis that sIL-6R levels have a causal relationship with depression. In additional exploratory analyses, we assess the robustness of the primary analysis and use Mendelian Randomization of other related proteins (C-reactive protein (CRP) and soluble glycoprotein 130 (sgp130)) to explore which IL-6 signaling pathway might explain the relationship.

2 Materials and Methods

2.1 Study design

This study uses a two-sample Mendelian Randomization design, in which information about the relationship between the genetic variants and the exposure (circulating levels of sIL-6R) is obtained from an existing published genome wide association study (GWAS).[42] We then apply the regression coefficients and standard errors for the genotype-exposure variable relationship to the genotype and outcome (depression) data from the second sample with a similar ethnic background.[42] This two-sample approach makes it possible to examine a relationship between an exposure and an outcome even when a large sample measuring both traits in the same individuals is not available.[43]

2.2 Main analysis samples

We obtained coefficients for the genotype/sIL-6R association from two studies, to allow for replication of results across samples. The first study was van Dongen et al 2014[44], a GWAS of 4,846 Dutch participants that measured sIL-6R using an ELISA assay. We also used the IMPROVE cohort GWAS[45] of 3,394 participants from multiple European countries which measured several proteins using an Olink array. Both studies were selected because the samples did not contain British participants and were therefore unlikely to overlap with the UK Biobank sample.

We conducted GWAS to calculate coefficients for the genotype/depression association using data from the UK Biobank.[46] Using this data, we defined the phenotype “recurrent depressive symptoms” based on self-reported symptoms. To allow for replication across depression samples, we obtained additional coefficients for the genotype/depression association from the Psychiatric Genomics Consortium meta-analysis of Major Depressive Disorder (PGC MDD 2018).[47] The version of the summary statistics used in our analysis does not include data from 23andMe, producing a final sample size of 59,851 cases and 113,514 controls.

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

The samples and phenotypes used in the analysis are shown in Table 1, and additional details are provided in Table S1 and Figures S1–S4.

2.3 Mendelian Randomization methods

We conducted Mendelian Randomization using several different methods: the Wald ratio of coefficients method,[48,49] the two-sample maximum likelihood method,[50] GSMR,[51] and

PCA-IVW.[52] These methods differ in several important aspects including statistical power, requirements for instrumental SNP selection, and availability of diagnostic tests to check the Mendelian Randomization requirements, allowing the strengths and weaknesses of the selected methods to complement each-other. Consistency of results across multiple methods helps to confirm the robustness of the results and ensure that they do not result from biases particular to one Mendelian Randomization method.[53,54]

For the single-SNP analysis using the Wald ratio of coefficients method,[48,49] we selected the biallelic SNP rs2228145, which explains approximately 51% of the variance in sIL-6R levels, making it a strong instrumental variable for Mendelian Randomization.[44] In datasets where information for rs2228145 was not available, we used the SNPs rs4129267 and rs12126142 as proxies, because they have r^2 values greater than 0.99 with rs2228145 in UK Biobank and in the 1000 Genomes EUR population. With the Wald ratio of coefficients method, the causal effect estimate is produced by dividing coefficient for the association between the instrumental SNP and the outcome ($\beta_{Y|Z}$) by the coefficient for the association between the instrumental SNP and the exposure ($\beta_{X|Z}$),[49] as shown in Figure 2.

We also used the two-sample maximum likelihood method,[50] which combines information from multiple independent SNPs to produce a causal effect estimate. We used multiple methods to select independent SNPs, which are discussed further in the supplemental note. In order to make sure the effects of rs2228145 could be easily examined in visual plots, we ensured selection of this SNP (or its best-available proxy) by excluding other SNPs in close LD with it prior to SNP selection. All analyses were performed in R 3.6.0 using the package TwoSampleMR 0.5.4. We used additional diagnostics to ensure the quality and consistency of the results. These included MR-Egger regression to check for SNPs that had an association with the outcome through a mechanism other than the exposure,[55] Cochran's Q to test for heterogeneity in per-SNP estimates of the odds ratio, and leave-one-SNP-out analyses to confirm that no single SNP produced large changes in the estimated causal effect.

Mendelian Randomization analyses using selected independent SNPs are sensitive to the specific SNPs used in the analyses, particularly when only a small subset of all eligible SNPs can be selected.[52] To address this limitation, we used two methods that can account for LD, PCA-IVW[52] and GSMR,[51] to allow for inclusion of a greater number of SNPs and to improve the statistical power of the analysis. GSMR can account for moderate levels of LD, allowing for a more lenient LD clumping threshold, while PCA-IVW uses principal components and eliminates the need for LD-based SNP selection entirely. The GSMR analysis was performed using GCTA 1.92.2 beta, with r^2 clumping thresholds ranging from 0.05 to 0.2 (Table 2). During this analysis, we used the HEIDI-outlier test to exclude any SNPs detected to be outliers or to potentially violate the Mendelian Randomization pleiotropy assumption.[51] The PCA-IVW analysis was performed in R 3.6.0 using code from Appendix A of Burgess (2017).[52] For the PCA-IVW analysis, we included all SNPs having at least a suggestive association with the exposure ($p < 1 * 10^{-6}$) and obtained data for the SNP correlation matrix using the UK Biobank sample and GCTA 1.92.2 beta.

2.4 Exploring potential mechanisms underlying IL-6 signaling and depression

The strongest SNP in the main analysis, rs2228145, has two potential effects on IL-6 signaling: the minor allele reduces signaling via the classical pathway, and under some circumstances might also be able to increase signaling via the trans pathway due to increasing levels of IL-6 and sIL-6R (Figure 1). There are two mechanisms through which rs2228145 could reduce classical signaling: buffering of IL-6 by increased availability of sIL-6R and reduced availability of mIL-6R due to increased shedding as sIL-6R.[34,35] Other genetic variants that increase sIL-6R levels may have differing effects on classical signaling - while they could still contribute to buffering effects from sIL-6R, their mechanisms of action may or may not involve shedding and decreased availability of mIL-6R. Unfortunately, the extremely strong effects of rs2228145 allow it to act as a confounder for other nearby SNPs, even with relatively low levels of linkage disequilibrium, which can lead to difficulty examining effects of other *IL6R* SNPs independent from the effects of rs2228145.

2.5 Exploratory analysis: Samples

The exploratory analyses used samples from the main analysis and three additional studies. We used results from the Framingham Heart Study[56] for sgp130, and results from the KORA study[57] for CRP. Although both studies had results for both proteins available, neither had a sufficient number of eligible significant SNPs for the other protein to use for replication Mendelian Randomizations. For replication of both proteins, we selected the INTERVAL study.[58] Because the INTERVAL study was based in the UK, there is potential for overlap with UK Biobank, which has the potential to bias Mendelian Randomization analysis.[59] However any overlap (if present) is likely to be small, and any bias that resulted would not also occur in the analyses using the Framingham and KORA data.

2.6 Exploratory analysis: Approach

First, we conducted an analysis using CRP as a proxy for classical IL-6 signaling (i.e. signaling via the membrane receptor, which stimulates CRP production).[35] We selected CRP as a proxy for classical IL-6 signaling because classical IL-6 signaling is the mechanism through which IL6R SNPs are likely to influence CRP levels.[35] A similar approach has been used in other Mendelian Randomization studies, which confirmed that *IL6R* variants produce results which differ from those of *CRP* variants in the expected direction.[41]

Second, in addition to using r^2 to assess LD between each SNP and rs2228145, we also examined Lewontin's |D'| statistic[60] because |D'| is not as severely affected by differences in allele frequency and may detect LD in some cases where r^2 does not (illustrated in Figure S5).[61] We then attempted to exclude the effects of rs2228145 by conducting additional Mendelian Randomization analyses using only SNPs having both $r^2 \leq 0.01$ and $|D'| \leq 0.15$ with rs2228145. We checked whether these SNPs still showed signs of affecting classical signaling by using them to perform Mendelian Randomization for CRP.

Third, we conducted a Mendelian Randomization analysis examining soluble glycoprotein 130 (sgp130), a protein which inhibits only trans IL-6 signaling.[18] If the trans signaling pathway were the mechanism for the causal relationship, higher levels of sgp130 might have

a protective effect against depression. The gene that encodes sgp130 (*IL6ST*) also encodes a membrane-bound form of gp130 that is used in both IL-6 signaling pathways, so SNPs in *IL6ST* have the potential to influence both pathways. To examine this possibility, we created a scatter plot to check whether per-SNP associations with sgp130 and with depression differed for SNPs from non-*IL6ST* genes.

All exploratory analyses used the PCA-IVW method so that all eligible SNPs could be included.

2.7 Ethical approval

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

3 Results

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

In the main analysis (Table 2), across all combinations of samples and methods, the majority of associations were significant, indicating that higher levels of sIL-6R were associated with increased odds of depression. For example, using the PCA-IVW method with the van Dongen and UK Biobank samples, a 10^{-8} g/mL increase in sIL-6R was associated with 1.023 times higher odds of depression (95% Confidence Interval: 1.006 – 1.039, p=0.006). Furthermore, even analyses which did not reach significance produced odds ratios greater than 1.0 (consistent in direction with the significant results). The consistency of the findings across the various combinations of exposure and outcome samples and across analytic methods indicates that the results are robust to differences in samples and analytic methods. We then repeated the main analysis using the “recurrent DSM-V major depression” phenotype, which is a more stringent definition but produces a smaller sample size because it can only be evaluated in participants who completed the UK Biobank Online Mental Health supplement. Despite this smaller sample, most analyses still produced significant or near-significant results (Table S4), and the direction of all odds ratios remained consistent and greater than 1.0.

We obtained similar results when using SNP coefficients from the van Dongen (2014) conditional analysis that estimated SNP coefficients for sIL-6R while adjusting for rs2228145 (shown in Table 2 as “PCA-IVW (conditional)”). Although the effect estimates from the analyses using the conditional GWAS coefficients were not statistically significant, the direction and magnitude of the estimated ORs was consistent with the other results in Table 2. However, conditional GWAS coefficients may not fully account for LD with a SNP that has strong effects[62,63] and Figure S10 shows patterns consistent with residual confounding by rs2228145.

We conducted a series of analyses to explore whether the identified causal relationship between IL-6 signaling and depression occurs via the classical or the trans signaling

pathway. First, we performed Mendelian Randomization for the effects of IL6R variants on CRP, which demonstrated that increased sIL-6R is associated with decreased CRP (Table S6). Next, we used these same variants to perform Mendelian Randomization for the effects of classical IL-6 signaling on depression, using CRP as a proxy for classical signaling. The results show that when using IL6R variants, higher CRP is associated with lower odds of depression (Table S7). Previous studies have shown that this finding is specific to *IL6R* variants and does not replicate when using variants that affect CRP via mechanisms other than classical IL-6 signaling.[41] These results demonstrate that reduced classical IL-6 signaling is one potential mechanism for the causal relationship sIL-6R and depression.

Table 3 extends the findings from Table 2 by repeating the Mendelian Randomization of sIL-6R with additional filtering to exclude the effects of rs2228145. Although the genetic instruments used in this exploratory analysis were not as strong as rs2228145, in several cases these analyses still suggested a relationship between sIL-6R and depression. In all filtered analyses producing significant or near-significant p-values, the effect estimates were above 1.0, illustrating that the relationship between sIL-6R and depression does not reverse direction when excluding the effect of rs2228145. We then used these same sets of filtered SNPs to perform Mendelian Randomization for the effects of sIL-6R on CRP (Table S6). The filtered variants no longer showed a significant causal effect on CRP, although it is not clear whether this results from a true lack of association or a reduction in statistical power from excluding the SNPs with the strongest effects.

The Mendelian Randomizations for sgp130 (Table S5) did not produce any statistically significant results, either when using variants only from the *IL6ST* gene or when including other variants associated with sgp130. Scatter plots (Figures S12 and S13) showed that the lack of correlation between SNP coefficients for sgp130 and for depression was not specific to the *IL6ST* gene, which suggests that the null finding is not attributable to opposing pleiotropic effects specific to *IL6ST* variants. The null result may indicate that the inhibitory effects of sgp130 on IL-6 trans signaling are not protective against depression. It is also possible that the null result occurred because the SNPs used in this analysis were not sufficiently strong instrumental variables. A recent paper has also suggested that rapid formation and dissociation of the IL-6/sIL-6R complex may limit sgp130's ability to inhibit trans signaling at typical concentrations,[39] which could mean that an sgp130 Mendelian Randomization may not reflect the effects of inhibiting the trans pathway.

4 Discussion

The results of the primary analysis are consistent with a causal effect of increased sIL-6R on risk of depression. This study used multiple Mendelian Randomization approaches to estimate this relationship, and the effect estimates were largely consistent regardless of the specific analytic approach used. These results build on existing cross-sectional[4–7] and longitudinal[8] studies suggesting a role for IL-6 signaling in depression. The results also strengthen evidence regarding the causal nature of the relationship between inflammation and depression by examining it in a manner that establishes both directionality and independence from environmental confounders.

While observational studies of inflammatory biomarkers and depression generally produce moderate effect estimates, the effect sizes estimated in this study were more modest. This discrepancy may reflect multiple factors. Observational studies often examine IL-6 itself, which may have larger and more direct effects than its receptor. In addition, depression is an etiologically heterogeneous condition, and inflammation may play a causal role in only a subset of cases.[64,65] Without a means of separating out those depression cases with an inflammation-related etiology, this heterogeneity could result in under-estimation of effect sizes for *IL6R* variants in genetic studies of depression.[66]

The results of this study suggest that one or more of the effects of increased sIL-6R has a causal relationship with depression. The most well-known effect of increased sIL-6R is a reduction of signaling via the classical pathway (as shown by the decrease in production of CRP). The direction of this effect is not consistent with the results of human and animal studies which suggest that depression is associated with higher, not lower, IL-6 levels and signaling activity. This inconsistency makes classical signaling less convincing as a causal mechanism, and suggests that other potential explanations should be examined.

The second potential mechanism is an increase in trans signaling. Although it has been predicted that increased availability of the soluble receptor would not increase trans signaling, the effects may vary across tissues and locations.[35] Typical concentrations of sIL-6R and sgp130 in cerebrospinal fluid (CSF) are considerably lower than those found in serum,[67] and *IL6R* genetic variants affect sIL-6R in CSF as well as serum.[68,69] When levels of sIL-6R and sgp130 are low, changes in sIL-6R might have the potential to influence trans signaling. rs2228145 explains 40% of the variance in sIL-6R in human CSF,[68,69] and animal studies have shown that spinal injection of sIL-6R can affect pain sensitivity,[70] which could support the idea that central nervous system concentrations of sIL-6R are low enough for receptor availability to influence trans signaling.[71] In addition, both observational[72,73] and experimental[74] studies have reported that depression is associated with increased IL-6 in CSF. This increased availability of IL-6 may allow sIL-6R availability to influence trans signaling, a phenomenon already observed in other scenarios involving elevated IL-6.[75,76] Although mechanisms involving the trans pathway offer an interesting explanation for our results, further study is needed to examine whether *IL6R* variants influence trans signaling in the brain and what conditions would be needed for such an influence to occur.

The complex biology of IL-6 signaling also introduces other possible explanations. Under certain circumstances the soluble IL-6 receptor may also act as a receptor for Ciliary Neurotrophic Factor,[77] although this mechanism would not explain the observational associations between IL-6 and depression. Additionally, a recently-discovered third type of IL-6 signaling known as trans-presentation is involved in Th17 cell differentiation in mice,[78] however little is currently known regarding this form of signaling in humans[79] or how *IL6R* genetic variants might affect it. Finally, even if a causal effect could be isolated to one specific IL-6 signaling pathway, this would not necessarily indicate a *direct* causal effect, because IL-6 signaling also impacts other biological pathways which may mediate its relationship with depression. Thus we consider it most appropriate to describe our result as supporting “a causal effect of increased sIL-6R on depression” and to regard the specific underlying pathway as an area still requiring further study.

As discussed in Lawlor 2016,[53] it is often helpful to use multiple study designs to "triangulate" an answer to complex causal questions. Animal studies have shown associations between IL-6 signaling and depression-like "sickness behaviors" as well as demonstrating that inhibition of trans signaling can reduce or prevent these symptoms.[25,26] Meanwhile, numerous observational studies in humans have reported associations between elevated IL-6 and depression,[4–6] and secondary analyses of clinical trials suggest that drugs which inhibit IL-6 signaling might have anti-depressant effects.[80,81] However, animal studies are limited by the use of animal behaviors as models for human depression, and observational studies and secondary analyses of trials are not suitable for causal inference. Thus despite its inability to identify the specific underlying mechanism, Mendelian Randomization of sIL-6R contributes an important piece to the puzzle by offering evidence to support a causal effect of sIL-6R on depression in humans.

4.1 Strengths and limitations

The primary limitation of this study is the presence of multiple potential causal mechanisms that could exist between sIL-6R and depression. Although this limitation prevents us from definitively identifying one specific mechanism as causal, results can still be interpreted as supporting a causal relationship between sIL-6R and depression without specifying a specific pathway. Additionally, sample overlap is likely between the van Dongen (2014) coefficients and the PGC MDD 2018 coefficients because both studies include individuals drawn from the Netherlands Twin Register (NTR) cohort. Such overlap may lead to bias,[59] however, we used multiple combinations of exposure and outcome samples to ensure that results were robust to overlap occurring between any specific pair of samples. Another limitation of this study is that the samples use similar, but not identical, phenotype definitions of depression. Our sIL-6R results replicated successfully in a supplementary analysis using a subset of UK Biobank participants who had data allowing for a phenotype definition that more closely resembled the clinical definitions used in PGC MDD 2018, but we did not use this subset as our main analytic sample due to the smaller sample size and resulting loss of statistical power. Finally, while of scientific interest, we did not attempt to use Mendelian Randomization to assess the potential causal effect of depression on IL-6 signaling for several reasons. Most salient is that unlike the direct effect of the *IL6R* variants on IL-6 receptors, variants associated with depression may have effects on the brain which simultaneously influence multiple psychiatric and behavioral phenotypes.[82] As a result, the Mendelian Randomization requirement that "the effect of the genetic instrument on the outcome must be mediated exclusively by the exposure in question"[33] precludes the examination of depression as an exposure until the biological mechanisms underlying genetic influences on depression are more fully understood.

This study also has several strengths, including the use of coefficients from large well-characterized samples (e.g., UK Biobank and PGC MDD 2018), analysis of multiple proteins and measures related to IL-6 signaling pathways, and consistency of results across multiple Mendelian Randomization approaches and samples. These findings help clarify the role of inflammation in the development of depression and suggest several avenues for future research that can inform efforts to both prevent and treat depression.

4.2 Conclusions

The findings from this study are consistent with a causal effect of increased sIL-6R on depression. Although we were not able to definitively isolate which of the IL-6 signaling pathways is the mechanism for the causal effect, examination of our results in combination with those produced by other studies provides some evidence to support the trans signaling pathway as a potential mechanism. These results should encourage further study of the effects of IL-6 signaling on depression, as well as encouraging exploration of drugs which modify IL-6 signaling as potential antidepressants.

Future research should examine the effects of rs2228145 and other *IL6R* variants on trans signaling in the human brain, and whether increasing sIL-6R levels in the brain can lead to increased susceptibility in animal models of sickness behavior or stress. Finally, the heterogeneous nature of depression[65] introduces several additional questions, including examination of possible gender-specific effects, identification of a subset of depression cases for whom IL-6 signaling might play a causal role, and assessment of whether the association may be stronger for depressive symptoms that more closely resemble the ones found in sickness behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Folkersen et al 2017 (DOI: 10.1371/journal.pgen.1006706) (IMPROVE Consortium)

Sun et al 2018 (DOI: 10.1038/s41586-018-0175-2) (INTERVAL Study)

Suhre et al 2017 (DOI: 10.1038/ncomms14357) (KORA cohort)

van Dongen et al 2014 (DOI: 10.1007/s10519-014-9656-8) (Netherlands Twin Register)

Wray et al 2018 (DOI: 10.1038/s41588-018-0090-3) (Psychiatric Genomics Consortium)

Yao et al 2018 (DOI: 10.1038/s41467-018-05512-x) (Framingham Heart Study)

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An earlier version of this analysis was included in KM Kelly's dissertation, which was submitted to the University of Michigan on August 17, 2020.

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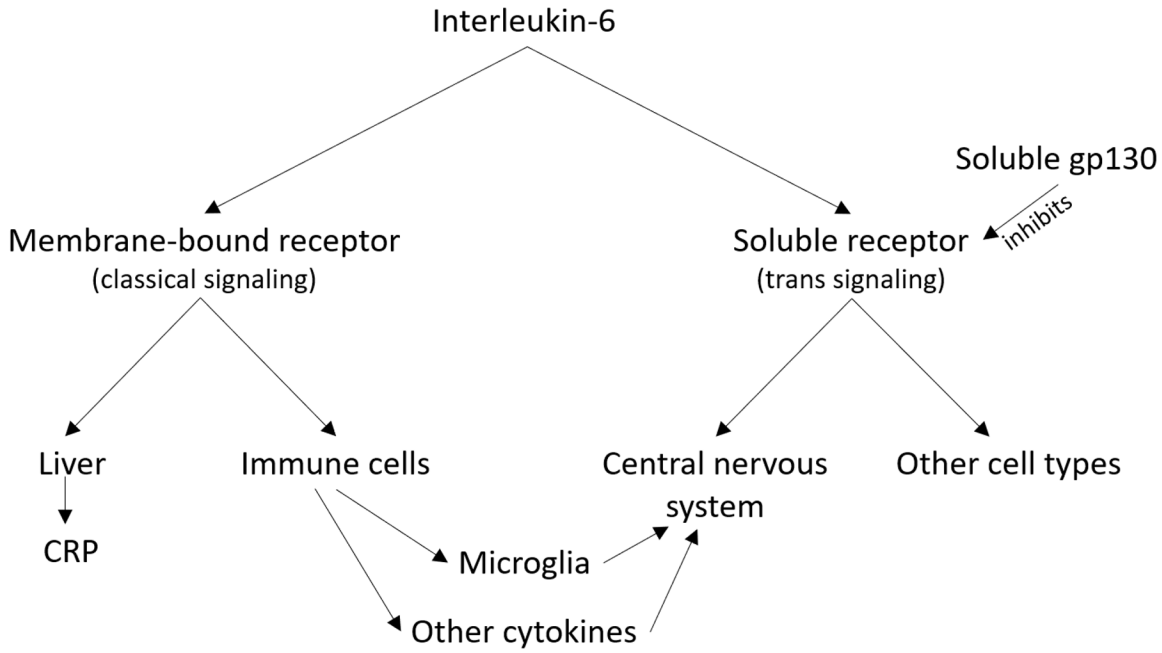

Figure 1:

Interleukin-6 signaling pathways

Figure 1 illustrates the two IL-6 signaling pathways. The trans signaling pathway is regarded as the more plausible mechanism for a relationship between IL-6 and depression due to the important role of trans signaling in the central nervous system. However, mechanisms exist through which classical signaling could influence the central nervous system, including the role of classical IL-6 signaling on immune regulation (which may influence other inflammatory signaling chemicals that then interact with the brain) and the presence of membrane IL-6 receptors on some microglia.

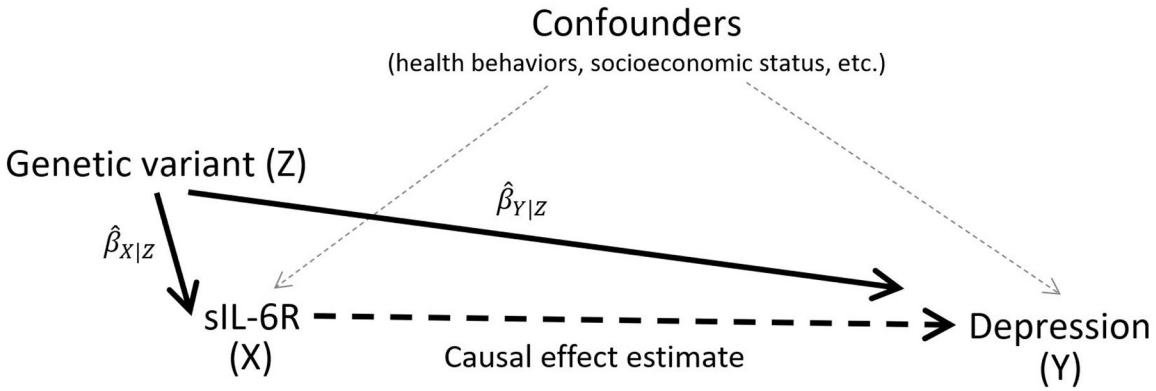

Figure 2:

The Mendelian Randomization study design

Figure 2 illustrates the Mendelian Randomization study design. The relationship between a genetic variant and the exposure (βx|z) and the relationship between a genetic variant and the outcome (βxy|z) are measured and used to estimate the causal effect of the exposure on the outcome independent of confounders.

Table 1:

Samples used in the analysis

Sample	Phenotype	N	Age range	Gender composition	Format
van Dongen 2014[44]	sIL-6R blood levels	4,846	18–90	61.3% female	GWAS coefficients
IMPROVE[45]	sIL-6R blood levels	3,394	55–79	Includes males and females, percent unknown	GWAS coefficients
UK Biobank[46]	Recurrent depressive symptoms	89,119	40–80	53.1% female	Individual-level data
PGC MDD 2018[47]	Major depressive disorder	173,005	Adults	Includes males and females, percent unknown	GWAS coefficients
INTERVAL[58]	C-reactive protein blood levels	3,301	Adults	41% male	GWAS coefficients
KORA[57]	C-reactive protein blood levels	997	32–81	Includes males and females, percent unknown	GWAS coefficients
Framingham[56]	sgp130 blood levels	5,257	Adults	53% female	GWAS coefficients

Table 2:

Results from sIL-6R Mendelian Randomization analyses

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

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

** Not enough SNPs to perform analysis

Table 2 shows the results of Mendelian Randomization analyses using two sIL-6R datasets (van Dongen 2014 and IMPROVE, shown on the left edge) and two outcome datasets (UK Biobank and PGC MDD 2018, shown across the top). The methods column shows both the analysis method (aligned left) and the SNP selection method (aligned right). For the Maximum Likelihood analysis, LD clumping was performed over a distance of 10,000 kilobases, and for the GSMR analysis clumping was performed using a 1 megabase window. For PCA-IVW analyses, the number appearing in parenthesis after the number of SNPs in the number of principle components (PCs) extracted from the SNP data to explain 99% of the variance.

Table 3:

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

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

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

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