

Inflammatory Biomarkers and Risk of Schizophrenia

A 2-Sample Mendelian Randomization Study

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IMPORTANCE Positive associations between inflammatory biomarkers and risk of psychiatric disorders, including schizophrenia, have been reported in observational studies. However, conventional observational studies are prone to bias, such as reverse causation and residual confounding, thus limiting our understanding of the effect (if any) of inflammatory biomarkers on schizophrenia risk.

OBJECTIVE To evaluate whether inflammatory biomarkers have an effect on the risk of developing schizophrenia.

DESIGN, SETTING, AND PARTICIPANTS Two-sample mendelian randomization study using genetic variants associated with inflammatory biomarkers as instrumental variables to improve inference. Summary association results from large consortia of candidate gene or genome-wide association studies, including several epidemiologic studies with different designs, were used. Gene-inflammatory biomarker associations were estimated in pooled samples ranging from 1645 to more than 80 000 individuals, while gene-schizophrenia associations were estimated in more than 30 000 cases and more than 45 000 ancestry-matched controls. In most studies included in the consortia, participants were of European ancestry, and the prevalence of men was approximately 50%. All studies were conducted in adults, with a wide age range (18 to 80 years).

EXPOSURES Genetically elevated circulating levels of C-reactive protein (CRP), interleukin-1 receptor antagonist (IL-1Ra), and soluble interleukin-6 receptor (sIL-6R).

MAIN OUTCOMES AND MEASURES Risk of developing schizophrenia. Individuals with schizophrenia or schizoaffective disorders were included as cases. Given that many studies contributed to the analyses, different diagnostic procedures were used.

RESULTS The pooled odds ratio estimate using 18 CRP genetic instruments was 0.90 (random effects 95% CI, 0.84-0.97; $P = .005$) per 2-fold increment in CRP levels; consistent results were obtained using different mendelian randomization methods and a more conservative set of instruments. The odds ratio for sIL-6R was 1.06 (95% CI, 1.01-1.12; $P = .02$) per 2-fold increment. Estimates for IL-1Ra were inconsistent among instruments, and pooled estimates were imprecise and centered on the null.

CONCLUSIONS AND RELEVANCE Under mendelian randomization assumptions, our findings suggest a protective effect of CRP and a risk-increasing effect of sIL-6R (potentially mediated at least in part by CRP) on schizophrenia risk. It is possible that such effects are a result of increased susceptibility to early life infection.

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[← Editorial page 1191](#)

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Neuropsychiatric disorders are major contributors to the burden of disease worldwide due to their substantial effect on disability.^{1,2} More than one-third of the top 25 global causes of disease burden in 2013, measured as years lived with disability, fall into this category of disorders.¹ Schizophrenia ranks 11th among the leading global causes of years lived with disability,¹ and its high burden is associated with substantial personal and societal costs.³

An association between schizophrenia and the immune system was suggested more than a century ago.⁴ Infections during pregnancy⁵ and early childhood,⁶ as well as autoimmune diseases,^{7,8} have been linked to an increased risk of schizophrenia. In addition, findings from the largest genome-wide association study of schizophrenia to date corroborate that immune dysregulation plays a role in the pathogenesis of schizophrenia.⁹ Inflammation has been hypothesized as a potential mechanism linking the immune response to the pathogenesis of schizophrenia and other neuropsychiatric disorders because cytokines may influence multiple neurologic processes, including neurotransmitter metabolism, neuroendocrine function, and neural plasticity.^{4,10-12} Observational epidemiologic studies, mainly of cross-sectional design, indicate that circulating levels of several cytokines,¹³ such as interleukin (IL)-1 β and IL-6, and C-reactive protein (CRP),^{14,15} are higher in individuals with schizophrenia. Meta-analysis of randomized clinical trials suggested that anti-inflammatory drugs improve the symptoms of the syndrome, but only a few studies with small sample sizes are available.¹⁶

Not only are there relatively few studies investigating the association between inflammatory biomarkers and schizophrenia in humans, they are largely of observational nature. Conventional observational studies may have important limitations, such as reverse causation and residual confounding,¹⁷⁻¹⁹ which hamper conclusions on whether specific anti-inflammatory agents could reduce the risk of developing schizophrenia. Genetic variants can be used as instrumental variables of modifiable exposures in a mendelian randomization (MR) design to improve the inference in observational studies. Justifications to rely on MR as a more robust method for causal inference than conventional observational studies include Mendel's laws and the fact that genotypes of germline genetic variation are defined at conception and are generally not associated with conventional confounders of observational studies.¹⁸⁻²⁰

Mendelian randomization has been used to investigate the effect of circulating CRP levels on schizophrenia risk. In a large, Danish population-based study using 4 genetic instruments in the *CRP* gene region, the point estimate suggested a risk-increasing effect, but the lower limit of the 95% CIs did not allow excluding the possibility of important protective effects.²¹ Two subsequent studies, both using the same summary association data sets in a 2-sample MR design, reported directionally inconsistent estimates,^{22,23} likely due to a data harmonization error in 1 of them.²⁴ Moreover, neither of the 2-sample MR studies performed substantial sensitivity analyses.

We conducted this study to investigate the effect of inflammatory markers on schizophrenia risk in a 2-sample MR design. We used genetic variants associated with inflammatory biomarkers as instrumental variables to improve infer-

Key Points

Question What is the effect of increased inflammatory biomarkers on the risk of developing schizophrenia?

Findings In this 2-sample mendelian randomization study using summary gene-biomarker association results estimated in pooled samples ranging from 1645 to more than 80 000 individuals, 2-fold increments in circulating levels of C-reactive protein and soluble interleukin-1 receptor levels were associated with a 10% reduction and a 6% increase in the lifetime odds of developing schizophrenia.

Meaning We found that blockade of interleukin-6 effects and low C-reactive protein levels might increase schizophrenia risk, possibly due to increased susceptibility to early life infection.

ence for a possible influence of the inflammatory biomarkers on risk of developing schizophrenia.²⁵

Methods

Data sets

We obtained summary association results for 4 sets of genetic instruments: liberal CRP (instruments selected using solely statistical criteria²⁶), conservative CRP (instruments restricted to the *CRP* gene region²⁷), interleukin-1 receptor antagonist (IL-1Ra),²⁸ and soluble interleukin-6 receptor (sIL-6R).²⁹ Summary associations between each instrument and schizophrenia risk were obtained from the largest schizophrenia genome-wide association study to date.⁹ The eMethods in the [Supplement](#) provides a description of each data set. Briefly, summary association results from large consortia of candidate gene or genome-wide association studies were included. Gene-biomarker associations were estimated in pooled samples ranging from 1645 to more than 80 000 individuals, while gene-schizophrenia associations were estimated in more than 30 000 cases and more than 45 000 ancestry-matched controls. In most studies included in the consortia, participants were of European ancestry, and the prevalence of men was approximately 50%. All studies were conducted in adults, with a wide age range (18 to >80 years). The summary genetic associations data sets were harmonized as described elsewhere²⁴ and are reported in eTable 1 and eTable 2 in the [Supplement](#). This project used only publicly available summarized (ie, aggregated) results from published meta-analyses of genome-wide association studies. Individual- and study-level aggregated results were not used.

Statistical Analysis

Single-nucleotide polymorphism (SNP) biomarker associations were collected in ln-transformed units. Odds ratio (OR) estimates of schizophrenia per 2-fold increments in circulating inflammatory biomarker levels were obtained as follows:

$$\left(e^{\sqrt{\text{OR}}}\right)^2$$

where OR is the odds ratio estimate per 1-ln increment in biomarker levels and *e* is the base of the natural logarithm.

Mendelian randomization requires that the genetic instruments are associated with the modifiable exposure of interest (assumption 1), and any association between the instruments and the outcome is mediated by the exposure (assumption 2). Given that only assumption 1 is empirically verifiable, careful consideration of potential violations of assumption 2 (due to factors such as population stratification, linkage disequilibrium, canalization, or horizontal pleiotropy) is important to minimize bias.¹⁸ An SNP that violates these assumptions is referred to as an invalid instrumental variable and its inclusion in MR analyses may bias the results.

Five MR methods were used.

1. Ratio method. This method was used to obtain individual SNP estimates by dividing the SNP schizophrenia by the corresponding SNP biomarker effect estimates. Standard errors were estimated using the delta method³⁰ assuming the uncertainty in the SNP-exposure association estimates was negligible (the no measurement error [NOME] assumption); this method corresponds to dividing, for each SNP, the SE of SNP schizophrenia association by the absolute value of the SNP biomarker effect estimate. These SEs were then used to perform weighted analyses using methods 2 to 4.
2. Inverse variance weighting (IVW). The IVW estimate is the inverse variance weighted mean of ratio estimates from 2 or more instruments.²⁵ This method assumes that all SNPs are valid instruments or are invalid in such a way that the overall bias is zero. We performed both fixed and multiplicative random effects IVW since the fixed effects method may be overprecise in the presence of heterogeneity that can occur due to, among other factors, horizontal pleiotropy or, more simply, off-target genetic effects.³¹
3. Weighted generalized linear regression. This method is similar to the IVW method but allows accounting for the correlation between the genetic instruments.³² The weighted generalized linear regression method was used instead of the IVW method when utilizing the conservative set of CRP genetic instruments, which comprised variants in partial linkage disequilibrium (eTable 3 in the [Supplement](#)).
4. Weighted median. The weighted median estimate is the median of the weighted empirical distribution function of individual SNP ratio estimates. It differs from a simple median estimate because the weight of each SNP in the overall estimate depends on the precision of its ratio estimate. More specifically, 50% of the weights in the analysis come from ratio estimates smaller than or equal to the weighted median. This method provides a consistent effect estimate if more than 50% of the information comes from valid SNPs.³³
5. Mendelian randomization Egger regression. MR Egger regression consists of a weighted linear regression of SNP schizophrenia against SNP biomarker effect estimates. Assuming that horizontal pleiotropic effects and SNP exposure associations are uncorrelated (ie, the instrument strength independent of direct effects assumption), MR Egger regression provides a valid effect estimate even if all SNPs are invalid instruments. Moreover, the MR Egger intercept can be interpreted as a test of overall unbalanced horizontal pleiotropy because one would expect a null y-intercept (ie, the mean value of the SNP schizophrenia

associations when the SNP biomarker association is zero) if there are no horizontal pleiotropic effects.³⁴ Both fixed and multiplicative random effects versions of the MR Egger regression method were performed.

Measurement error in the SNP exposure associations (ie, NOME violation) is always present to some degree. In the 2-sample setting (which is the case in our study), NOME violation attenuates the effect estimates and also affects MR Egger regression intercept. The degree of NOME violation in IVW and MR Egger regression, respectively, can be quantified by the following statistics³⁵:

$$\frac{\bar{F}_{GX} - I}{\bar{F}_{GX}} \text{ and } I_{GX}^2.$$

Both range from 0% to 100% and can be interpreted as the amount of attenuation in the effect estimates due to NOME violations.^{35,36} Such violations can be accounted for using the simulation extrapolation method, which was applied to MR Egger regression.³⁶

The Cochran Q test for heterogeneity was applied to the liberal CRP set to test for the presence of horizontal pleiotropy. This test assumes that all valid genetic instruments estimate the same effect.³⁷ Moreover, to identify potentially influential instruments in the liberal CRP set of instruments, we applied a range of influence tests (described in detail in the eMethods in the [Supplement](#)).^{38,39} Tests of influence were complemented by a leave-1-out approach to evaluate the influence of each SNP.

We used available data on the association between the sIL-6R genetic instrument and CRP levels²⁹ in a mediation analysis evaluating the potential mediating effect of CRP in the association between sIL-6R levels and schizophrenia risk. We used MR to obtain effect estimates of the exposure-outcome (ie, sIL-6R levels and schizophrenia risk), exposure-mediator (ie, sIL-6R levels and CRP levels), and mediator-outcome (ie, CRP levels and schizophrenia risk) associations. The exposure-mediator and mediator-outcome associations can be used to estimate the expected effect of sIL-6R levels on schizophrenia risk assuming that CRP levels fully mediate this association. This effect estimate can then be contrasted to the observed exposure-outcome association to gain insights into the mediating effect of the putative mediator (the calculation is described in detail in the eMethods in the [Supplement](#)).⁴⁰

Analyses were performed using R, version 3.2.4 (<http://www.r-project.org>).

Results

Table 1 displays the association between genetically elevated inflammatory biomarkers and schizophrenia. Regarding CRP, when the liberal set of 18 CRP-associated variants was used, results were consistent among the 3 MR methods, with ORs of schizophrenia of 0.90 (random effects 95% CI, 0.84-0.97), 0.91 (95% CI, 0.85-0.98), and 0.93 (random effects 95% CI, 0.82-1.05) per 2-fold increment in circulating CRP

Table 1. Odds and Odds Ratios of Schizophrenia per 2-Fold Increments in Inflammatory Markers Based on MR

SNP Set and MR Method	Parameter	OR or Odds (95% CI)	P Value
CRP, liberal (n = 18) ^a			
IVW (FE)	OR	0.90 (0.86-0.95)	<.001
IVW (RE)	OR	0.90 (0.84-0.97)	.005
MR Egger (FE)	Intercept (odds)	1.00 (0.99-1.00)	.42
	OR	0.93 (0.85-1.01)	.09
MR Egger (RE)	Intercept (odds)	1.00 (0.99-1.01)	.57
	OR	0.93 (0.82-1.05)	.21
Weighted median	OR	0.91 (0.85-0.98)	.02
CRP, conservative (n = 4) ^b			
Ratio: rs1130864	OR	0.92 (0.81-1.05)	.21
Ratio: rs1205	OR	0.92 (0.84-1.01)	.06
Ratio: rs1800947	OR	0.90 (0.79-1.02)	.10
Ratio: rs3093077	OR	0.97 (0.84-1.12)	.69
WGLR	OR	0.93 (0.86-1.00)	.04
IL-1Ra (n = 2)			
Ratio: rs1542176	OR	1.02 (0.98-1.07)	.35
Ratio: rs6743376	OR	0.98 (0.94-1.01)	.20
IVW (FE)	OR	0.99 (0.83-1.19)	.73
IVW (RE)	OR	0.99 (0.76-1.30)	.82
sIL-6R (n = 1)			
Ratio: rs222814	OR	1.06 (1.01-1.12)	.02

Abbreviations: CRP, C-reactive protein; FE, fixed effects; IL-1Ra, interleukin-1 receptor antagonist; IVW, inverse variance weighting; MR, mendelian randomization; OR, odds ratio; RE, random effects; sIL-6R, soluble interleukin-6 receptor; SNP, single nucleotide polymorphism; WGLR, weighted generalized linear regression.

^a The liberal CRP set corresponds to the SNPs identified by Dehghan and colleagues.²⁶

^b The conservative CRP set corresponds to the SNPs analyzed by Wensley and colleagues.²⁷

levels using IVW, weighted median, and MR Egger regression approaches, respectively (Figure). The following statistical results:

$$\frac{\bar{F}_{GX} - 1}{\bar{F}_{GX}} = 99.8\% \text{ and } I_{GX}^2 = 98.3\%$$

suggest that measurement errors in the SNP CRP associations were not substantially attenuating the effect estimates. Regular and simulation extrapolation-corrected MR Egger regression results were virtually identical, so only the first result was shown.

The MR Egger intercepts were (when rounding to 2 decimal digits) equal to 1.00 with narrow 95% CIs, suggesting no strong unbalanced horizontal pleiotropy. However, the Cochran Q statistic was 31.9, with an associated P value of .02, suggesting some heterogeneity in the effect estimates, possibly due to horizontal pleiotropy.

The results obtained using the conservative CRP set were consistent with the liberal set. Odds ratio estimates of schizophrenia per 2-fold increment in circulating CRP levels based on the ratio method ranged from 0.90 (95% CI, 0.79-1.02) to 0.97 (95% CI, 0.84-1.12). The pooled OR estimate was 0.93 (95% CI, 0.82-1.05). The 2 OR estimates for IL-1Ra were directionally inconsistent (1.02 [95% CI, 0.98-1.07] and 0.98 [95% CI, 0.94-1.01]), with a pooled IVW estimate of 0.99 (random effects 95% CI, 0.76-1.30). Finally, the OR of schizophrenia per 2-fold increments in circulating sIL-6R levels was 1.06 (95% CI, 1.01-1.12).

In the leave-1-out MR analyses using the liberal CRP set, all OR estimates of schizophrenia per 2-fold increment in circulating CRP levels were directionally consistent (Table 2). Inverse variance weighting, weighted median, and MR Egger re-

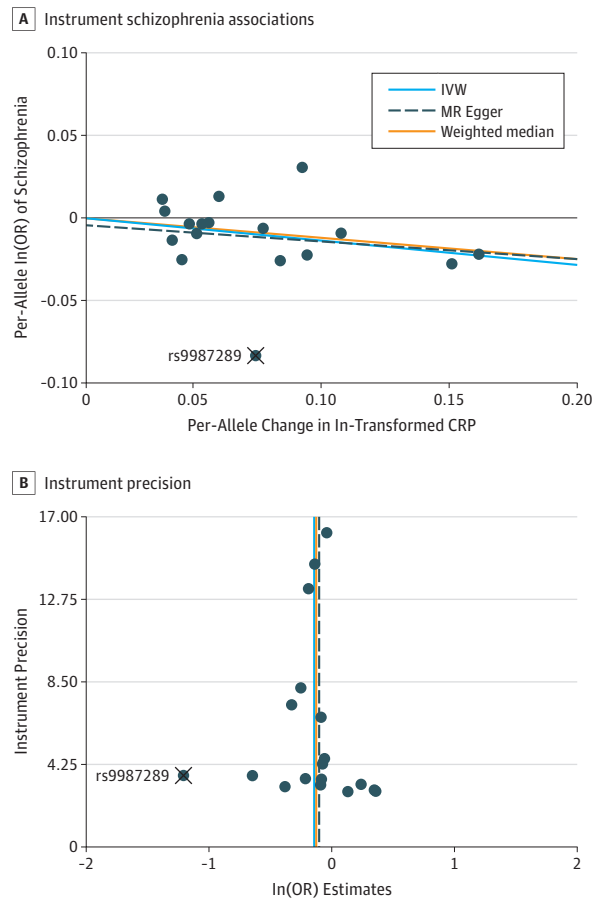
gression estimates ranged from 0.88 to 0.91, 0.89 to 0.94, and 0.87 to 0.94, respectively. Conventional statistical significance levels were achieved in all IVW estimates and in 16 weighted median estimates but in none of the MR Egger regression estimates. The SNP rs9987289 was the only variant classified as potentially influential, and its removal had virtually no effect on the results. This single variant accounted for most of the heterogeneity in the individual instrument ratio estimates (eTable 4 in the Supplement). Therefore, any possible horizontal pleiotropy suggested by the Cochran Q test does not explain our findings.

Using the ratio method, the effect of a 1-unit increase in ln(sIL-6R) levels (used as a proxy for inhibition of IL-6 classic signaling) on ln(CRP) levels was -0.26 (95% CI, -0.32 to -0.21), and the effect of a 1-unit increase in ln(CRP) levels on the ln (OR) of schizophrenia was -0.14 (95% CI, -0.23 to -0.05). Multiplying these estimates yielded an OR of schizophrenia of 1.03 (95% CI, 1.01 to 1.04) per 2-fold increment in sIL-6R levels. The estimated proportion of the effect of inhibiting IL-6 classic signaling on schizophrenia risk that is mediated by CRP (using the IVW estimate from the liberal CRP set as the effect estimate of CRP on schizophrenia risk) was 43.8% (95% CI, 3.3% to 84.2%).

Discussion

We used 2-sample MR to evaluate the association between inflammatory biomarkers and schizophrenia. Overall, we did not find strong evidence that lifelong exposure to increased action of these proinflammatory cytokines increases schizophrenia risk, as previously hypothesized, and, conversely, found

Figure. Mendelian Randomization (MR) Analyses Regarding the Effect of C-Reactive Protein (CRP) Levels on Schizophrenia Using the Liberal Set of 18 Genetic Instruments



A, Instrument schizophrenia associations (y-axis) against instrument CRP associations (x-axis). B, Instrument precision (ie, instrument CRP regression coefficients divided by the correspondent instrument schizophrenia SEs) (y-axis) against individual instrument ratio estimates in log odds ratio of schizophrenia (x-axis). In(OR) indicates odds ratio estimate per 1-ln increment in biomarker levels; IVW, inverse variance weighting; X, the single genetic instrument classified as influential (rs9987289).

that blockade of IL-6 effects and low CRP levels might instead increase schizophrenia risk. Moreover, part of the association between IL-6 signaling and schizophrenia might be mediated by CRP levels, which is consistent with previous knowledge on the major role of IL-6 in inducing acute-phase response and the fact that lower CRP levels are a downstream effect of inhibiting IL-6 classic signaling.^{26,41,42} For IL-1Ra, point estimates were inconsistent between instruments, and 95% CIs were large. Our study extends previous MR findings by evaluating different inflammatory markers and applying a range of sensitivity analyses.

Interleukin-6 is known for its pivotal role in integrating immune response, such as by inducing hepatic acute-phase proteins, differentiation of T cells, and tissue regeneration.⁴³ Apart from being a sensitive marker of systemic inflammation and tissue damage, CRP is an acute-phase protein that contributes to host defense against infection.⁴⁴ C-reactive pro-

tein binds to phosphocholine expressed on the surface of cells and some bacteria, which activates the complement system, promoting phagocytosis and clearance of necrotic and apoptotic cells and bacteria.^{44,45}

Mechanisms underlying the association of blockade of IL-6 classic signaling and lower CRP levels with increased risk of schizophrenia are unknown. We speculate that they relate to increased susceptibility to early life infection. Blockade of IL-6 classic signaling leads to increased susceptibility to infections in mice⁴⁶ and humans.⁴³ Observational studies indicate that low levels of some acute-phase proteins in newborns were related to higher schizophrenia risk⁴⁷ and that neonates who develop schizophrenia later in life have an impaired capacity of increasing levels of acute-phase proteins, such as CRP, in response to some maternal infections compared with controls.⁴⁸ In adults, prospective studies indicate that higher CRP levels are related to increased susceptibility to infections.^{49,50} However, these findings should be interpreted cautiously as higher CRP levels may reflect subclinical infection, chronic activation of the inflammatory response, pre-existing disease, and socioeconomic or lifestyle characteristics. As an example, several types of infection (ie, HIV as well as hepatitis A, B, and C) appear to be more prevalent among individuals with mental disorders, including schizophrenia, in multiple settings.⁵¹ An MR study reported that genetic predisposition to higher CRP levels was not associated with increased infection risk in adults.⁵⁰ To our knowledge, no existing MR study has investigated IL-6 and CRP effects on early life infection risk.

Causal inference from MR relies on some assumptions, 1 of them being that the exposure completely mediates any association between the instruments and the outcome. Most of the genetic instruments that we used have biological justifications for their selection, except for the liberal CRP set of 18 variants. Half of the variants in the liberal CRP set have been associated with 1 or more of 10 tested cardiometabolic phenotypes, while the other variants were not. Among the first half of the variants, 6 (or other variants indexing the same locus) were associated with CRP levels independently of their association with cardiometabolic phenotypes, which was not the case for the remaining 3 variants.⁴² Although these findings suggest that the CRP-associated SNPs in the liberal set are pleiotropic, our results based on these variants were consistent among the 3 MR methods (which have different assumptions regarding horizontal pleiotropy) and were corroborated by the results for the conservative CRP set and the leave-1-out analysis. The latter is also important because the liberal CRP set included the IL-6R variant **rs4129267**, which was not used as a genetic instrument for IL-6 classic signaling but might nevertheless influence it. Moreover, previous studies using the conservative CRP set, the IL-1Ra, and the IL-6R instruments observed that, in general, those variants are not associated with conventional confounders.^{27,29,52}

The SNP biomarker and SNP schizophrenia estimates were obtained in mostly European studies, thus minimizing the possibility of population stratification bias. This similarity also increases the plausibility of the 2-sample MR assumption that summary genetic association results were obtained in samples from

Table 2. Odds Ratios of Schizophrenia per 2-Fold Increments in CRP Levels Based on MR Using the Liberal Set of 18 CRP-Associated Variants in a Leave-1-Out Approach

Excluded SNP	IVW (RE)		MR Egger (RE)		Weighted Median	
	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
rs10521222	0.90 (0.84-0.97)	.007	0.93 (0.82-1.05)	.23	0.91 (0.85-0.97)	.02
rs10745954	0.91 (0.85-0.97)	.007	0.91 (0.81-1.04)	.15	0.91 (0.86-0.98)	.02
rs1183910	0.91 (0.84-0.98)	.02	0.94 (0.82-1.08)	.37	0.94 (0.87-1.01)	.10
rs12037222	0.90 (0.84-0.97)	.008	0.93 (0.81-1.06)	.23	0.91 (0.86-0.98)	.02
rs12239046	0.90 (0.84-0.97)	.007	0.93 (0.82-1.06)	.25	0.91 (0.85-0.97)	.02
rs1260326	0.90 (0.84-0.97)	.007	0.93 (0.82-1.05)	.23	0.91 (0.85-0.97)	.01
rs13233571	0.90 (0.84-0.96)	.005	0.93 (0.82-1.05)	.24	0.91 (0.85-0.97)	.02
rs1800961	0.90 (0.84-0.96)	.005	0.93 (0.82-1.05)	.21	0.91 (0.85-0.98)	.02
rs2794520	0.90 (0.83-0.98)	.01	0.93 (0.80-1.08)	.30	0.94 (0.87-1.01)	.13
rs2847281	0.90 (0.84-0.96)	.005	0.94 (0.83-1.07)	.35	0.91 (0.85-0.97)	.02
rs340029	0.90 (0.84-0.97)	.006	0.94 (0.82-1.07)	.30	0.91 (0.85-0.97)	.01
rs4129267	0.91 (0.85-0.98)	.01	0.93 (0.82-1.05)	.23	0.92 (0.86-0.99)	.03
rs4420065	0.91 (0.84-0.98)	.01	0.93 (0.82-1.06)	.25	0.92 (0.86-0.99)	.04
rs4420638	0.88 (0.81-0.95)	.002	0.87 (0.74-1.02)	.08	0.89 (0.83-0.95)	.004
rs4705952	0.90 (0.84-0.97)	.007	0.93 (0.82-1.06)	.25	0.91 (0.85-0.97)	.02
rs6734238	0.90 (0.84-0.97)	.007	0.93 (0.82-1.06)	.25	0.91 (0.85-0.97)	.01
rs6901250	0.90 (0.84-0.97)	.008	0.92 (0.81-1.05)	.21	0.91 (0.85-0.98)	.02
rs9987289 ^a	0.91 (0.87-0.96)	.002	0.92 (0.84-1.01)	.09	0.91 (0.86-0.98)	.02

Abbreviations: CRP, C-reactive protein; IVW, inverse variance weighting; MR, mendelian randomization; OR, odds ratio; RE, random effects; SNP, single nucleotide polymorphism.

^a This variant was classified as potentially influential both in IVW and MR Egger.

the same or comparable populations. Regarding power, although some SNP biomarker associations were estimated in small samples, the statistical evidence for association in such data sets was generally strong. Moreover, power in the 2-sample setting depends more on the precision of the SNP outcome than on the SNP exposure association,²⁵ and SNP schizophrenia associations were estimated in approximately 80 000 individuals.

Limitations

Interpreting the magnitude of estimates for the effect of CRP and IL-6 on schizophrenia risk, as well as for the mediated effect of IL-6 by circulating CRP, requires caution. Our MR analysis likely reflects lifelong exposure to elevated cytokine and CRP levels. However, it is possible that only exposure to IL-6 and CRP in a specific window of time (eg, early life) affects schizophrenia risk. We obtained estimates for the SNP-cytokines and SNP-CRP associations from adults, but these associations might differ in early life. In addition, we used estimates for the effect of the IL-6R genetic instrument on sIL-6R as a proxy to investigate the total and the indirect (mediated by CRP) effect of blocking IL-6 classic signaling. However, this genetic instrument affects IL-6 classic signaling by increasing cleavage of membrane-bound IL-6R, which results in lower availability of membrane-bound IL-6R and higher availability of sIL-6R. Both mechanisms are likely to contribute to in-

hibiting IL-6 classic signaling.⁴³ Finally, it is possible that IL-6 and CRP effects on schizophrenia risk are related to a maternal effect (eg, maternal susceptibility to infections during pregnancy), so that our findings are explained by the correlation between maternal and offspring genotypes. Because maternal and offspring genotypes are correlated, any effect of intrauterine exposures or maternal behavior influenced by the mother's genetic background would also result in an association between the offspring's genotype and risk of schizophrenia.⁵³

Conclusions

Our findings support the notion that lower CRP levels and blockade of IL-6 cell signaling—both associated with lower inflammation and acute phase response—increase schizophrenia risk. This finding suggests that the positive associations of CRP and IL-6 with schizophrenia risk in conventional observational studies are due to limitations, such as reverse causation or residual confounding. Even though our findings could be a result of horizontal pleiotropy that we failed to detect and account for, they at least suggest that increased levels of inflammatory biomarkers do not lead to substantially higher schizophrenia risk.

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