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RESEARCH ARTICLE

Genetically proxied IL-6 signaling and risk of Alzheimer's disease and lobar intracerebral hemorrhage: A drug target Mendelian randomization study

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Abstract

INTRODUCTION: Evidence suggests that higher C-reactive protein (CRP) is associated with lower risk of Alzheimer's disease (AD) and lobar intracerebral hemorrhage (ICH). Whether interleukin (IL)-6 signaling, an active pharmacological target upstream of CRP, is associated with these amyloid-related pathologies remains unknown.

METHODS: We used 26 CRP-lowering variants near the IL-6 receptor gene to perform Mendelian randomization analyses for AD (111,326 cases, 677,663 controls) and ICH (1545 cases, 1481 controls). We explored the effect of genetically proxied IL-6 signaling on serum, cerebrospinal fluid (CSF), and brain proteome (971 individuals).

RESULTS: Genetically upregulated IL-6 receptor-mediated signaling was associated with lower risk of AD (OR per increment in serum logCRP levels: 0.87, 95% CI: 0.79–0.95) and lobar ICH (OR: 0.27, 95% CI: 0.09–0.89). We also found associations with 312, 77, and 79 brain, CSF, and plasma proteins, respectively, some of which were previously implicated in amyloid-clearing mechanisms.

DISCUSSION: Genetic data support that CRP-lowering through variation in the gene encoding IL-6 receptor may be associated with amyloid-related outcomes.

KEYWORDS
Alzheimer's disease, amyloid, C-reactive protein, IL-6, intracerebral hemorrhage, Mendelian randomization

Evangelos Pavlos Myserlis and Anushree Ray contributed equally to this work.

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Highlights

- Genetic variants proxying IL-6 inhibition are associated with AD and lobar ICH risk.
- The variants are also associated with amyloid clearing-related proteomic changes.
- Whether pharmacologic IL-6 inhibition is linked to AD or lobar ICH merits further study.

1 | BACKGROUND

Alzheimer's disease (AD) and cerebral amyloid angiopathy (CAA) implicate mechanisms that involve amyloid accumulation in the brain and blood vessel walls.[1] Recent evidence from observational and genetic data in European individuals suggests that lower circulating C-reactive protein (CRP) levels at midlife are associated with a higher risk for AD and all-cause dementia.[2] We previously also demonstrated that genetic variants in the locus of the *CRP* gene leading to lifelong decreases in circulating CRP are associated with higher lobar intracerebral hemorrhage (ICH) risk, a phenotype commonly associated with CAA.[3] While the mechanisms underlying these associations remain unclear, it has been postulated that CRP may be involved in the opsonization and phagocytosis of amyloid beta (Aβ) by microglia and the activation of the complement system; thus, reduced CRP levels may lead to ineffective clearance of Aβ.[2] These data could raise concerns about the potential effect of drugs reducing CRP levels on amyloid clearance.

CRP itself is not the direct target of any existing drugs. However, interleukin (IL)-6 signaling regulates CRP production and has been the target of both approved and under-development treatments.[4] Inhibitors of the IL-6 receptor, such as tocilizumab and sarilumab, are already in use in clinical practice for the treatment of autoimmune disorders[5–7] and in critically ill patients with COVID-19,[8] although long-term safety to make inferences about possible associations with cognitive outcomes are missing. Furthermore, agents targeting the IL-6 pathway have gathered attention due to the recent evidence supporting their potential for atheroprotection. Data from the Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial have demonstrated that IL-1β inhibition reduces the rate of cardiovascular events[9] and this reduction is proportional to reductions in circulating levels IL-6 and high-sensitivity CRP.[10] As such, ziltivekimab, an antibody targeting IL-6, was tested in a phase 2 study, where it showed efficacy in lowering CRP levels up to 90%[11] and is now actively being tested in a phase 3 trial for lowering the burden of cardiovascular events.[12] Interestingly, the brain anti-inflammatory effects of canakinumab are also explored in a phase 2 clinical trial for patients with mild cognitive impairment or mild AD.[13]

Given that IL-6 is upstream to CRP and strategies targeting IL-6 or its receptor have been shown to considerably lower CRP levels, the genetic findings connecting CRP to amyloid-related pathologies raise concerns about whether this might represent a potential side-effect. Previously, we detected genetic variants in the locus, including the gene encoding the receptor of IL-6, that show comparable effects to pharmacological targeting of the IL-6 signaling pathway and, as such, represent proxies for studying its effects in silico.[13–18] Here, we use genetic data to explore whether genetically proxied IL-6 signaling is associated with AD and lobar ICH using drug target Mendelian randomization (MR) analyses. Furthermore, we aimed to detect potential proteomic mediators in the plasma, cerebrospinal fluid (CSF), and brain with the overarching aim to identify the mechanisms underlying these associations.

2 | METHODS

2.1 | Study design

An overview of our study design is provided in Figure 1. We selected genetic variants proxying the effects of IL-6 signaling and ran Mendelian randomization analyses on amyloid-related outcomes. We also investigated the effects of genetically proxied IL-6 signaling on serum, CSF, and brain proteome with the goal of detecting proteins that potentially mediate an effect on amyloid-related outcomes.

2.2 | Selection of genetic instruments

2.2.1 | Genetically proxied IL-6 signaling

For our analyses, we used a drug-target MR approach, which has some methodological differences compared to a traditional genome-wide MR analysis.[19] Within the drug-target MR framework, genetic instruments are selected based on their location in the genome according to a specific drug target, rather than at the genome-wide level, and are weighed based on a reliable downstream effect, which could include a relevant phenotype, biomarker, or gene expression or protein levels. To recapitulate the effects of IL-6 signaling, we selected variants that lie within the *IL6R* gene or a region 300 kb upstream or downstream and are associated with genetically proxied circulating CRP levels, a downstream biomarker of IL-6 signaling utilizing a methodology that has been previously described.[15] Briefly, we selected variants that were independent ($r^2 < 0.1$) and significantly ($p < 5 \times 10^{-8}$) associated with serum CRP, in the abovementioned genomic region, from a genome wide association study (GWAS) of 522,681 individuals in the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.[20] A list of the variants is provided in Table S1. The

F-statistics for each single nucleotide polymorphism (SNP) included in genetic instruments for univariable MR analyses were calculated using the formula $F = \beta^2/SE^2$.[21] The conditional *F*-statistics for each exposure included in multivariable MR analyses were calculated using the strength_mvmr function in the MVMR R package.

2.2.2 | Outcome data

We used two main outcomes to proxy amyloid-related pathology: lobar ICH and AD. We leveraged publicly available GWAS data for our outcomes. Specifically, for ICH, we utilized data from a GWAS of 1545 ICH cases (664 lobar ICH and 881 non-lobar ICH) and 1481 ICH-free controls,[22] and for AD, we utilized a GWAS of 111,326 clinically diagnosed/"proxy" AD cases and 677,663 controls.[23] As an additional analysis, to increase specificity in our findings, we also utilized a GWAS case-control study of late-onset AD that did not include proxy AD cases in the case definition (17,008 AD cases vs. 37,154 controls).[24]

2.2.3 | Proteome-wide MR analysis

To explore the effect of IL-6 signaling on the proteome of three different compartments, we performed two-sample MR analyses utilizing data for 869 proteins in 971 CSF samples, 953 proteins in 636 plasma samples, and 1296 proteins in 458 brain samples from patients with and without AD and cognitively normal individuals of European ancestry and another study including measurements of 4907 proteins from 35,559 plasma samples using an aptamer-based platform.[25,26] To explore proteins that possibly mediate the effect of IL-6 signaling on amyloid-related pathology, we followed a mediation MR approach.

2.3 | Statistical analysis

After selecting genetic variants for IL-6 signaling as described above, we performed two-sample MR analyses on our two main outcomes, AD and lobar ICH. We used the inverse variance (IVW) approach as the main methodology in our analysis.[27] We performed sensitivity analyses utilizing the weighted median estimator, which allows for some pleiotropic variants,[28] as well as the MR-Egger approach, which is less powered but generates robust estimates even in the presence of pleiotropy for all variants included in the genetic instrument.[29] Utilizing multiple MR sensitivity approaches and deriving consistent effect size estimates across methodologies provides further support of the validity of the main MR approach, although, inherently these approaches may not be as well powered as IVW to detect a statistically significant association.[30] In order to explore the possibility of reverse causation, we also performed reverse MR, exploring the effect of genetic predisposition to AD and lobar ICH on circulating IL-6 levels. We first generated genetic instruments to proxy AD and lobar ICH, by extracting genome-wide significant ($p < 5 \times 10^{-8}$), independent ($r^2 < 0.01$) variants associated with these traits, utilizing GWAS summary statistics from the aforementioned studies. Then, we performed univariable IVW MR on circulating IL-6 levels, leveraging data from a GWAS meta-analysis of circulating cytokines that included up to 8293 Finnish individuals.[31]

To further investigate the effect of the IL-6 signaling at the proteome-wide level and explore proteins that possibly mediate the effect of IL-6 signaling on amyloid-related pathology, we followed a mediation MR approach.[32] We first explored associations of IL-6 signaling with CSF, plasma, and brain proteins using IVW MR analyses. To determine differences in the MR associations across different compartments, we calculated absolute differences in beta estimates of each compartment-pair for each protein. We selected proteins significantly associated with IL-6 after correcting for multiple hypothesis testing at a false discovery rate (FDR) of <0.05. Thereafter, we determined the multivariable MR effect of each significant protein and IL-6 signaling on AD and ICH. The mediation effect of each protein on the association between Il-6 and disease outcome was calculated as the difference between the beta estimates of univariable and multivariable MR analyses between Il-6 and disease outcomes divided by the beta estimate of the univariable analyses. Standard errors (SEs) were estimated using the delta method. For proteins with a mediation effect of >10%, we extracted *cis*-acting genetic instruments ($p < 1 \times 10^{-5}$, $r^2 < 0.1$) that lie within 300 kb from the gene that encodes for the respective protein and used those variants to perform univariable IVW MR analyses with AD as an outcome. All analyses were performed in R studio version 3.6.1.[33]

RESEARCH IN CONTEXT

1. **Systematic review**: Our MEDLINE search yielded evidence suggesting that genetic variation in the *CRP* gene leading to decreased circulating C-reactive protein (CRP) levels is associated with higher risk of Alzheimer's disease (AD) and lobar intracerebral hemorrhage (ICH), commonly attributed to cerebral amyloid angiopathy. Whether IL-6 signaling, an active pharmacological target upstream of CRP, is associated with these amyloid-related pathologies has not been systematically explored.
2. **Interpretation**: Genetic variants mimicking IL-6 signaling downregulation were associated with higher risk of AD and lobar ICH, as well as proteomic changes previously implicated in amyloid-clearing mechanisms. Thus, CRP-lowering through IL-6 receptor signaling downregulation may be associated with amyloid-related outcomes.
3. **Future directions**: Future research should experimentally replicate the role of IL-6 signaling in brain amyloid clearance. Clinical studies should explore whether current or under-development pharmacological IL-6-targeting approaches could have a clinically relevant effect on amyloid-related outcomes.

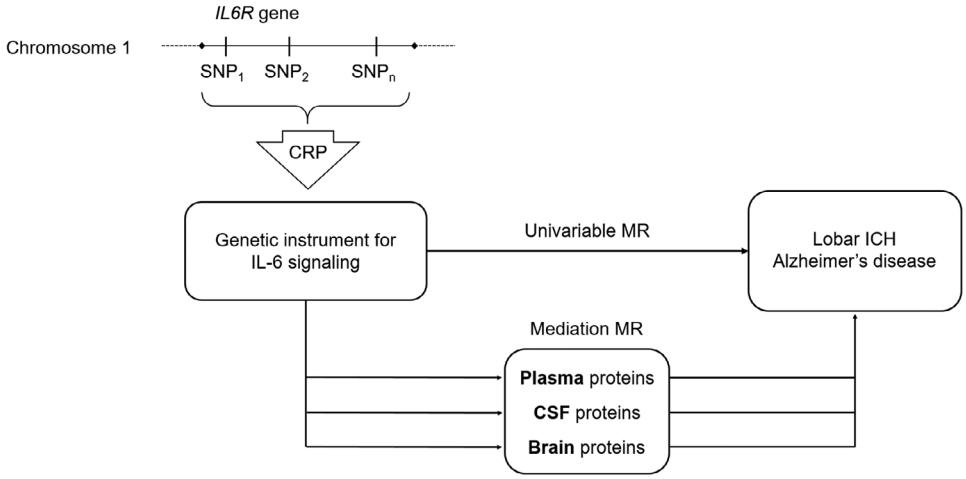

FIGURE 1 Study design. We selected genetic variants that are associated with circulating CRP levels and that lie within the *IL6R* region to proxy the effects of IL-6R mediated signaling and ran Mendelian randomization analyses on amyloid-related outcomes. We also investigated the effects of genetically proxied IL-6 signaling on serum, CSF, and brain proteome with the goal of detecting proteins that potentially mediate an effect on amyloid-related outcomes. CRP, C-reactive protein; CSF, cerebrospinal fluid; ICH, intracerebral hemorrhage; IL, interleukin; MR, Mendelian randomization; SNP, single nucleotide polymorphism.

3 | RESULTS

3.1 | Associations between genetically proxied IL-6 signaling, AD, and ICH

Using 26 *cis*-acting CRP-lowering variants in the vicinity of *IL6R*, we found genetically upregulated IL-6R signaling to be associated with a lower risk of AD (odds ratio [OR] per increment in serum logCRP levels: 0.87, 95% confidence interval [CI]: 0.79–0.95) and lobar ICH (OR: 0.27, 95% CI: 0.09–0.89), but not non-lobar ICH (OR: 1.21, 95% CI: 0.41–3.54), suggesting that IL-6 signaling may be implicated in amyloid-clearing mechanisms (Figure 2). Results for AD and lobar ICH were directionally consistent, albeit not significant, in sensitivity analyses utilizing alternative MR methods (MR-Egger and weighted median) that account for possible pleiotropic effects of included variants (Table S2), as well as in analyses that did not include AD-by-proxy cases in the definition (Table S3). In bidirectional MR analyses, genetic predisposition to AD was not associated with circulating IL-6 levels. We could not explore the effect of a genetic proxy for risk of lobar ICH given that no genome-wide significant variants were present for this phenotype (Tables S4 and S5).

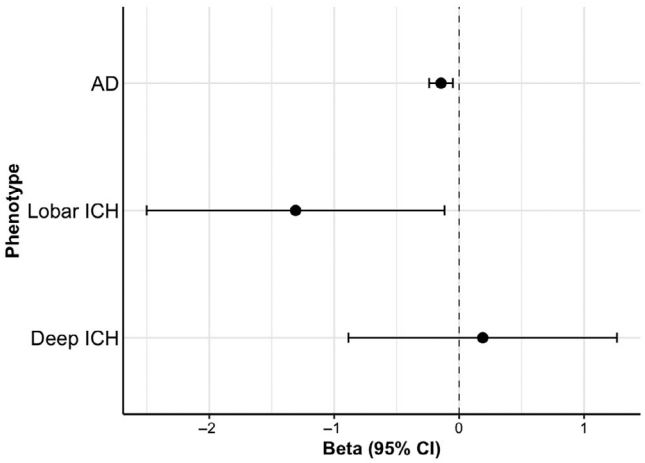

FIGURE 2 Mendelian randomization estimates of the associations between genetically proxied IL-6 signaling, Alzheimer's disease, and intracerebral hemorrhage. The beta correspond to 1-log increment in serum C-reactive protein levels. AD, Alzheimer's disease; CI, confidence interval; ICH, intracerebral hemorrhage; IL, interleukin.

3.2 | Associations between genetically proxied IL-6 signaling and plasma, CSF, and brain proteomic changes

Next, in order to detect potential protein mediators of these associations, we tested the effects of genetically proxied IL-6 receptor signaling on 1296 proteins in the brain, 869 in the CSF, and 953 in the plasma (Tables S6-S8). We found significant associations (FDR-corrected p-value < 0.05) with 312 proteins in the brain, 77 proteins in the CSF, and 79 proteins in the plasma (Figure 3A). However, only the significant association with IL-6 receptor subunit alpha was replicated in the additional plasma proteome dataset (Table S9). There were only weak correlations in the proteomic effects of genetically proxied IL-6 signaling across plasma, CSF, and plasma (Figure 3B), suggesting tissue- and compartment-specific proteomic signatures. Among others, the effect of IL-6 signaling across the three different compartments was most distinct for IL-6 receptor subunit alpha, which increased as a result of genetic downregulation of the pathway only in CSF and plasma and 14-3-3 protein family, which decreased as a result of genetic IL-6 signaling downregulation (Table S10). Of the significantly associated proteins, 20 proteins in the brain, three in the CSF, and 32

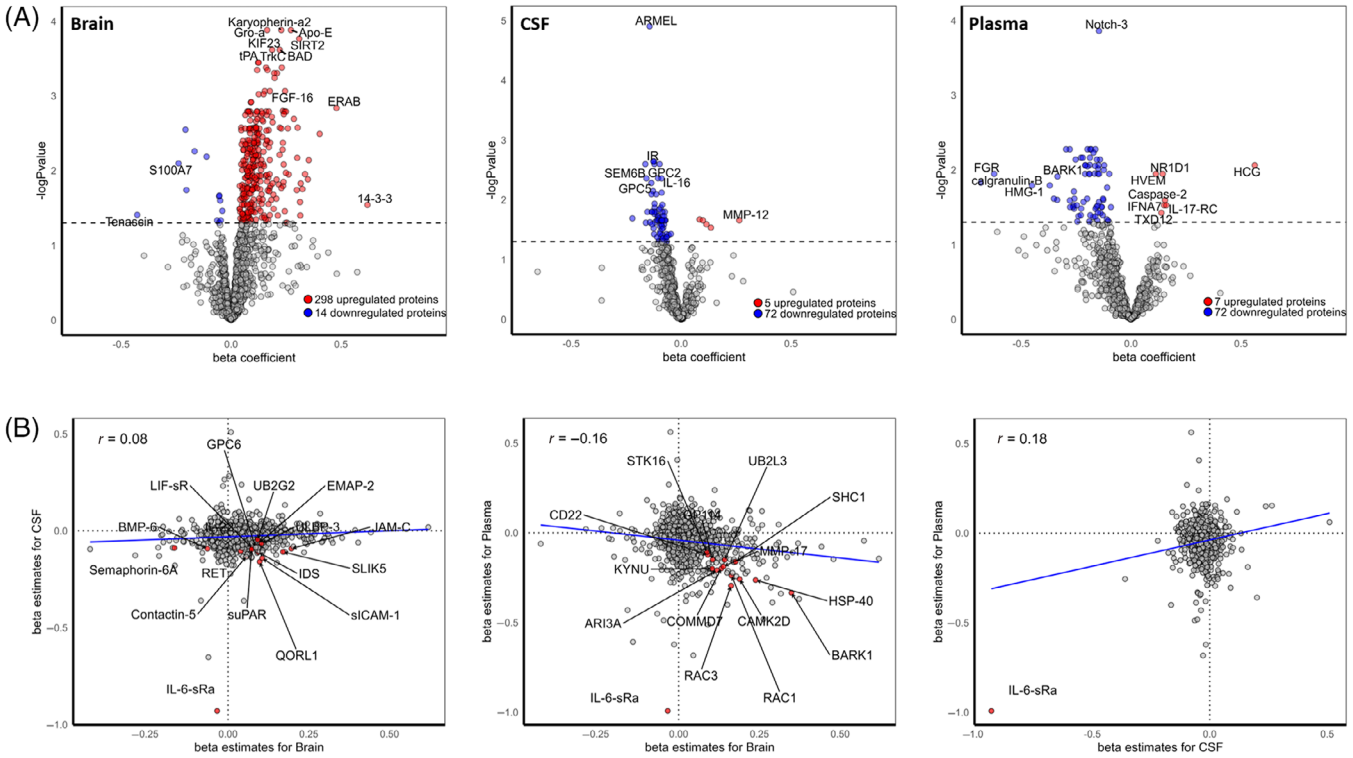

FIGURE 3 Association between genetically proxied IL-6 signaling and proteomes of brain, CSF, and plasma. (A) Volcano plots of the results of Mendelian randomization analyses between IL-6 signaling and the proteomes of brain, CSF and plasma. The dashed horizontal line corresponds to a false discovery rate (FDR)-corrected p-value < 0.05. (B) Scatter plots showing correlation in the Mendelian randomization estimates of proteins associated with IL-6 signaling between each pair of compartments. The red dots correspond to proteins that were found to be significantly associated (FDR-corrected p-value < 0.05) with IL-6 signaling in both tissues. CSF, cerebrospinal fluid; IL, interleukin.

in the plasma showed a mediation effect of >10% in multivariable MR analyses for AD (Table S11). Among these proteins, leukemia inhibitory factor receptor (LIFR) and the 14-3-3 protein family in the brain, which were positively associated with genetically proxied IL-6 receptor signaling, have been previously demonstrated to have a protective role against AD.[34,35] Similarly to upstream to IL-6R molecules in the IL-6 signaling cascade that are compensatorily upregulated as a result of genetic downregulation of the pathway, the negative correlation with LIFR could also represent a compensatory effect, given the known feedback loop between IL-6 and LIF signaling.[36] On the other hand, intercellular adhesion molecule 1 (ICAM-1) in the CSF and eukaryotic translation initiation factor 4 gamma 2 (EIF4G2) in the plasma, which were negatively associated with IL-6 receptor signaling, have been shown to promote AD pathogenesis.[36–38] *Cis*-acting genetic instruments within the predetermined thresholds could only be obtained for three proteins in the CSF and six proteins in the plasma (Table S12). We found no directionally consistent significant associations between genetically proxied proteins and the risk of AD.

4 | DISCUSSION

In this drug target MR analysis, we found that genetically proxied IL-6 receptor-mediated signaling, an upstream regulator of CRP and active therapeutic target, is associated with amyloid-related clinical outcomes, specifically with AD and lobar ICH. Additionally, we investigated the proteomic effects of genetically proxied IL-6 signaling on plasma, CSF, and brain and found significant associations with proteins in biological pathways that are involved in AD pathogenesis. These results could have implications in the setting of pharmacologic targeting of IL-6 signaling.

Converging evidence from clinical trials and MR studies suggest that targeting inflammation through IL-6 inhibition may lower cardiovascular risk. Data from the CANTOS trial showed that the effect of pharmacologic inhibition of IL-1β leads to a proportional decrease in IL-6 and hs-CRP, and MR analyses suggest that targeting IL-6R signaling may lead to reductions in cardiovascular risk.[16] However, when repurposing existing drugs that may influence inflammation or developing new drug targets, off-target effects have to be taken into account.[16] Our current work suggests that pharmacologic inhibition of IL-6 may be linked to increases in AD and lobar ICH risk possibly through amyloidogenic pathways, as demonstrated in our proteome-wide association analysis. LIFR, which was negatively associated with genetically proxied IL-6 receptor signaling, is involved in LIF signaling that attenuates Aβ-induced neuronal cytotoxicity.[34] On the other hand, ICAM-1, which was positively associated with IL-6 receptor signaling, is upregulated in response to Aβ in vitro and has an increased expression in Aβ plaques.[37,38] Further, IL-6 receptor signaling was found to be

associated with decreased levels of EIF4G2, which is involved in the translation initiation of circular RNA circAb-a forming polypeptide Ab175, which can be processed into Aβ peptides.[39] It has previously been shown that elevations in CRP are inversely associated with the burden of Aβ in the cortex in apolipoprotein E (*APOE*) ε4 carriers, suggesting that inflammation may exert a beneficial effect on early stages of AD by Aβ amyloid plaque microglia phagocytosis.[40] We should also not exclude the possibility that the effect of genetically proxied IL-6 signaling on risk of AD and lobar ICH might be mediated by non-amyloidogenic pathways, such as tau spreading, blood-brain barrier disruption, or neuroinflammation.[41] Furthermore, previous research has shown that higher levels of soluble IL-6 receptor in the plasma and CSF are associated with worse neurocognitive outcomes.[42] Our results are in line with and provide further context to these findings, as previous data have indicated that genetically proxied IL-6 inhibition is associated with higher soluble IL-6 receptor levels.[15]

Concurrently, the potential harmful off-target effect of IL-6 inhibitors in the brain that our data are suggestive of, has to be taken into the context of an overall positive impact that the same agents might have on cerebrovascular health through cardioprotective mechanisms. We should acknowledge that it would be difficult to infer the contribution of genetically proxied IL-6 signaling to dementia given the bidirectional effects on risk of AD and atherosclerotic cardiovascular disease. According to neuropathological studies, the majority of dementia cases represent a mixture of AD and vascular pathologies. It would be important to also consider the impact in patients homozygous for *APOE* ε4, among whom higher levels of CRP have been linked to reduced Mini-Mental State Examination (MMSE) scores.[43] While our GWAS did not specifically stratify participants based on vascular contributions to cognitive impairment and dementia (VCID) or *APOE* ε4 status, these factors are crucial and warrant further investigation to understand their impact on IL-6 inhibition outcomes. Future studies should incorporate these variables to elucidate the nuanced effects of IL-6 targeting in AD patients with comorbid vascular conditions. Although our results suggest that lowering IL-6 signaling may increase AD and lobar ICH risk, a differential effect may exist on this risk depending on whether drugs are able to pass the blood-brain barrier, so further research on animal models or intermediate markers is needed.

Our work has limitations. First, the main weakness of this work is the inherent limitation of *cis*-MR analyses to exploring lifelong effects of a specific target, and therefore, the magnitude of the estimates may not be correlated with short-term perturbations in the same target. Only data from clinical trials testing treatment with inhibitors of IL-6 signaling could answer this concept. However, future genetic analyses in individual-level data could also explore the impact of genetically proxied IL-6 signaling on AD endophenotypes across different age groups. Second, we used genomic data from European populations, potentially limiting the transferability of our inferences across other ancestries. Third, the pleiotropic effects of SNPs used as genetic instruments for proteins on neighboring genes could not be ruled out, despite the choice of a 300 kb window upstream or downstream of the gene encoding the respective protein. Fourth, although we identified signals as potential proteomic mediators for the association between IL-6 inhibition and the examined outcomes, our analyses are limited by power, and other signals that were not well-powered enough may exist.

In conclusion, we found that genetically proxied IL-6 downregulation may be associated with AD and lobar ICH risk. Our genetic data should be interpreted with caution and further experimental studies are needed to replicate these findings and explore any clinically relevant effect that IL-6 targeting may have on AD and lobar ICH.

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CONFLICT OF INTEREST STATEMENT

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CONSENT STATEMENT

For all our analyses, we utilized publicly available summary statistics GWAS data of the respective traits, and therefore no direct informed consent was necessary. Information regarding the informed consent process in the individual studies from which the data were obtained may be found in the respective manuscripts.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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