Systematic druggable genome-wide Mendelian randomisation identifies therapeutic targets for Alzheimer’s disease

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ABSTRACT

BACKGROUND Alzheimer’s disease (AD) is the leading cause of dementia. Currently, there are no effective disease-modifying treatments for AD. Mendelian randomisation (MR) has been widely used to repurpose licensed drugs and discover novel therapeutic targets. Thus, we aimed to identify novel therapeutic targets for AD and analyse their pathophysiological mechanisms and potential side effects.

METHODS A two-sample MR integrating the identified druggable genes was performed to estimate the causal effects of blood and brain druggable expression quantitative trait loci (eQTLs) on AD. A repeat study was conducted using different blood and brain eQTL data sources to validate the identified genes. Using AD markers with available genome-wide association studies data, we evaluated the causal relationship between established AD markers to explore possible mechanisms. Finally, the potential side effects of the druggable genes for AD treatment were assessed using a pheno-wide MR.

RESULTS Overall, 5883 unique druggable genes were aggregated; 33 unique potential druggable genes for AD were identified in at least one dataset (brain or blood), and 5 were validated in a different dataset. Among them, three prior druggable genes (epoxide hydrolase 2 (EPHX2), SERPINB1 and SIGLEC11) reached significant levels in both blood and brain tissues. EPHX2 may mediate the pathogenesis of AD by affecting the entire hippocampal volume. Further pheno-wide MR analysis revealed no potential side effects of treatments targeting EPHX2, SERPINB1 or SIGLEC11.

CONCLUSIONS This study provides genetic evidence supporting the potential therapeutic benefits of targeting the three druggable genes for AD treatment, which will be useful for prioritising AD drug development.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The rapid increase in the prevalence and societal and economic burden of Alzheimer’s disease (AD) has prompted an urgent need for efficient intervention and treatment for AD. Currently, there are no effective disease-modifying treatments for AD.

WHAT THIS STUDY ADDS

⇒ This study provides genetic evidence supporting the potential therapeutic benefits of targeting the three druggable genes (epoxide hydrolase 2 (EPHX2), SERPINB1 and SIGLEC11) for AD treatment and analyses their pathophysiological mechanisms and potential side effects.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Our study makes a significant contribution to the literature because we provide genetic evidence supporting the potential therapeutic benefits of targeting the three druggable genes for AD treatment, which will be useful for prioritising AD drug development.

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BACKGROUND Alzheimer’s disease (AD) is the most common neurodegenerative disease and is the leading cause of cognitive impairment and dementia.1 An estimated 6.3 million Americans aged ≥65 years are living with AD.2 This number might reach 13.8 million by 2060.3 As an age-dependent disease, AD is a threat to public health because of the rapid ageing of the world population. Over the past three decades, an increasing trend in the burden of AD has been observed globally.4 The rapid increase in the prevalence and societal and economic burden of AD has prompted an urgent need for efficient intervention and treatment for AD.4

However, AD treatment remains challenging. First, only a few drugs are available for AD.5 Second, most approved drugs exert their therapeutic effects by targeting only a few specific molecular targets. For example, almost all antidementia drugs exert their therapeutic effects by inhibiting cholinesterase enzyme or glutaminergic pathway.6 Third, in addition to their beneficial therapeutic effects, antidementia drugs have considerable side effects, including nausea, vomiting, loss of appetite, headache and constipation.7 Importantly, these antidementia drugs cannot cure or delay AD progression; they only provide symptomatic treatment.

Over the past few decades, great efforts have been made to explore disease-modifying treatments for AD; however, little progress has been achieved. Most agents targeting amyloid beta (Aβ), aggregated tau, or neurofibrillary tangles may delay disease progression, but there is no substantial evidence suggesting their clinical benefits.8 Therapeutic stasis is primarily attributed to unknown AD pathophysiology. Fortunately, large-scale
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human genetic studies provide an opportunity for novel drug development for many complex diseases because drug targets supported by genetic evidence have a greater chance of success in drug discovery pipelines. In other words, ‘druggable’ genes encoding proteins or gene expression can provide powerful clues to inform drug targets. Over the past few years, numerous large-scale genome-wide association studies (GWASs) have identified many single-nucleotide polymorphisms (SNPs) associated with AD risk. However, GWAS cannot clearly and directly provide clues regarding causal genes and drug targets because many identified SNPs are located in non-coding or intergenic regions.

Mendelian randomisation (MR) is an approach used to assess the causality between a modifiable exposure or risk factor and a clinically relevant outcome. MR analysis has been widely used to repurpose licensed drugs and discover novel therapeutic targets by integrating summary data from disease GWASs and expression quantitative trait locus (eQTL) studies. The expression levels of a gene may be considered as a kind of lifelong exposure, and eQTL located in the genomic region of the druggable genes are always considered as proxies.

Therefore, we performed a systematic druggable genome-wide MR to identify therapeutic targets for AD. First, a two-sample MR analysis was performed to estimate the causal effects of blood and brain druggable eQTLs on AD. Second, we performed colocalisation analyses to verify the robustness of the expression instrumental variables (IVs) and conducted a repeat study using different blood and brain eQTL data sources to validate the identified genes. Third, we evaluated the causal relationships between the identified genes and established AD markers to explore the possible mechanisms by which these genes are involved in AD pathogenesis. Finally, we assessed the potential adverse effects of the identified druggable genes for AD treatment using a phenome-wide MR.

MATERIALS AND METHODS
The overall study design is illustrated in figure 1. Further details of the methods and materials used are provided as follows.

Identification of druggable genes
Drugable genes were obtained from the Drug–Gene Interaction Database (DGIdb V.4.2.0, https://www.dgidb.org/) and a recent review on the ‘druggability’ of genes. The DGIdb provides information on drug–gene interactions and druggable genes

Figure 1 Overview of this study. First, we identified druggable genes. Second, a two-sample MR was conducted to estimate the causal effects of blood and brain druggable eQTLs on AD. We then performed colocalisation analyses to verify the robustness of the expressions’ IVs, and a repeat and validation study was conducted. Third, we aimed to identify AD biomarkers with GWAS data available. Subsequently, we evaluated the causal relationship of prior expressions on AD biomarkers to explore possible mechanisms. Finally, we assessed the potential side effects of targeting the prior druggable gene products for AD treatment via a phenome-wide MR.

AD, Alzheimer’s disease; CSF, cerebrospinal fluid; DGIdb, Drug–Gene Interaction Database; eQTL, expression quantitative trait locus; FA, fractional anisotropy; FDR, false discovery rate; GWAS, genome-wide association study; IV, instrumental variable; MD, mean diffusivity; MR, Mendelian randomisation; TSS, transcriptional start site.
from publications, databases and other web-based sources. We downloaded the ‘Categories Data’ (released in February 2022), including all genes in the druggable categories in the DGIdb, from all sources mapped to Entrez genes. We also obtained a list of druggable genes from a review by Finan et al.

### eQTL datasets

The discovery blood eQTL dataset was obtained from eQTLGen (https://eqtlgen.org/), where the cis-eQTLs of 16,987 genes were obtained from 31,684 blood samples of healthy European-ancestry individuals. Fully significant cis-eQTL results (false discovery rate (FDR) <0.05) and allele frequency information were obtained. The discovery brain eQTL data were obtained from the PsychENCODE consortia (http://resource.psychencode.org), which included 1387 prefrontal cortex and primarily European samples. We downloaded all significant eQTLs (FDR <0.05) for genes with expression of >0.1 fragments per kilobase per million mapped fragments in at least 10 samples and all SNP information. Further details on the data are presented in online supplemental table S1 and in the original publications.

In addition, we used whole blood and brain frontal lobe cis-eQTLs from the Genotype-Tissue Expression project (GTEx) V8 (https://gtexportal.org/home/datasets) to validate our findings. Descriptions of donor enrolment, consent process, biospecimen procurement, sample fixation and other information are presented in online supplemental table S1 and on the website (https://www.gtexportal.org).

### AD GWAS dataset

The outcome data used in this study were obtained from the most recent and largest AD GWAS, which included 111,326 clinically diagnosed or “proxy” AD cases and 677,663 controls. It comprises various European GWAS consortia already working on AD and a new dataset collated from 15 European countries. The subgroups included in this GWAS are presented in online supplemental table S2 and more information is available in the original publication. Moreover, a GWAS meta-analysis of only clinically diagnosed AD cases (stage I: 21,982 cases, 41,944 controls) was used to validate our findings (online supplemental table S2).

### eQTL MR analysis

MR analyses were performed using the R package TwoSampleMR V0.5.6. The eQTLs of the drug genome were selected as the exposure data. To generate IVs, we selected SNPs with FDR of <0.05 and within ±100 kb from each gene’s transcriptional start site (TSS). SNPs in each eQTL were then clumped at $r^2$ <0.01 using European samples from the 1000 Genomes Project. The R package PhenoScanner V1.0 was used to find IVs-related phenotypes. Furthermore, we removed SNPs that were directly related to the outcome data (AD) and AD-associated traits (e.g., dementia and family history of AD). The outcome data were then loaded and harmonised using the in-built functions. The Wald ratio method was used to compute the MR estimates for each SNP. Where more than one SNP was available, a weighted mean of the ratio estimates, weighted by the inverse variance of the ratio estimates (inverse-variance weighted, IVW), was used. We assessed whether the MR–Egger intercept significantly deviated from 0 to test horizontal pleiotropy when the number of SNPs is no less than three. Moreover, Cochran’s Q methods were used to test for heterogeneity between Wald ratios. FDR-corrected p values were calculated, and FDR of <0.05 was considered significant. In the replication studies, nominal p values of <0.05 were considered statistically significant.

### Colocalisation analysis

Occasionally, an SNP is located in two or more gene regions. In this case, if an SNP contained eQTL information for two or more different genes, its effect on the disease (here, AD) would be mixed by different genes, and colocalisation analysis was used to confirm that AD and the eQTLs may share causal genetic variants. Briefly, for significant MR results in the discovery, we performed a colocalisation analysis for AD risk and the SNP within ±100 kb from each gene’s TSS in eQTL via the R package COLOC V5.2.0 with $P_1 = 1×10^{-4}$, $P_2 = 1×10^{-4}$ and $P_3 = 1×10^{-3}$. The probability that a given SNP is linked to AD is denoted as $P_1$; the probability that a given SNP is a significant eQTL is denoted as $P_2$; and the probability that a given SNP is an outcome of both AD and eQTL is denoted as $P_3$. The COLOC package was used to test five hypotheses. The posterior probability (PP) was used to quantify support for all the hypotheses, and they were identified as PPH0–PPH5: PPH0, no association with either trait; PPH1, association with the expression of the gene but not the AD risk; PPH2, association with the AD risk but not the expression of the gene; PPH3, association with the AD risk and expression of the gene, with distinct causal variants; and PPH4, association with the AD risk and expression of the gene, with a shared causal variant. We restricted our analysis to genes reaching PPH1+PPH4 of ≥0.8 owing to limited power in the colocalisation analysis.

### Markers associated with AD

To determine whether these three AD-causal genes (epoxide hydrolase 2 (EPHX2), SIGLEC11 and SERPINB1) which we identified in our study via MR analysis were involved in the hypothetical pathological mechanism of AD, we first performed another set of two-sample MR with the same MR parameters between the three identified genes’ eQTL (EPHX2, SIGLEC11 and SERPINB1) in the brain and blood and the hypothetical biomarkers to investigate the effect of gene expression on AD biomarkers. We then performed the second step using the markers’ GWASs as exposures and the AD GWAS as the outcome to explore the mediating role. The biochemical and neuroimaging markers of AD were obtained from previous reviews (online supplemental table S3).

### Phenome-wide MR

To study the potential side effects of these three prior druggable genes, we used gene expression as exposure and summary statistics of diseases in the UK Biobank cohort (n≤408,961) as outcomes to perform phenome-wide MR. Disease GWASs from the UK Biobank were performed using the Scalable and Accurate Implementation of Generalised Mixed Model (SAIGE V0.29) method to account for unbalanced case–control ratios. Owing to statistical power, we chose 783 traits (diseases) with more than 500 cases for phenome-MR analyses. Summary statistics of disease-associated SNPs were downloaded from the SAIGE GWAS (https://www.leelabs.org/resources). Further details are provided in the publication. We then used the same parameters to perform MR analysis using the prior druggable gene eQTL in the blood via the IVW method. The causal effects are considered statistically significant at FDR<0.05.

### RESULTS

#### Druggable genome

Using the data available in DGIdb v4.2.0, we identified 3953 genes as potentially druggable (online supplemental table S4).
In addition, we extracted 4463 genes with druggability from a review\(^4\) (online supplemental table S5). Finally, 5883 unique druggable genes with Human Genome Organisation Gene Nomenclature Committee names were aggregated from the two sources mentioned above for further analysis (online supplemental table S6).

**Candidate druggable genes for AD**

We used eQTLs from the brain tissue and blood to intersect with druggable genes to obtain druggable eQTLs. All primitive significant brain eQTLs (FDR <0.05) included 14653 gene symbols after removing SNPs beyond ±100 kb from the TSS. When overlapping with the druggable genome, we obtained the brain druggable eQTL as the exposure, which included 4422 gene symbols. After the SNPs were clumped, 3021 genes remained to run the MR. After removing the SNPs directly related to AD, 21 candidate druggable genes for AD were identified following FDR adjustment (FDR <0.05) using the Wald ratio or the IVW method (figure 2). The IVs for significant expression, PhenoScanner outcome, and full results of the MR are presented in online supplemental table S7–9.

Regarding blood eQTL, by running criteria similar to the brain eQTL, blood druggable eQTL exposure, including 2860 gene symbols, was obtained to perform MR. As a result, we found 12 potential drug targets (figure 2 and online supplemental table S10). In summary, 33 unique potential druggable genes for AD were identified in at least one dataset (brain and blood). Notably, three prior druggable genes (EPHX2, SERPINB1 and SIGLEC11) reached significant levels in the blood and brain tissues. They all showed risk influence on AD (in the brain, EPHX2 OR 1.27, SERPINB1 OR 1.41 and SIGLEC11 OR 1.12; in the blood, EPHX OR 1.10, SERPINB1 OR 1.14 and SIGLEC11 OR 1.10, respectively).

Subsequently, we performed a colocalisation analysis to further determine the probability that SNPs associated with AD and eQTL shared causal genetic variants using the SNP within ±100 kb from the TSS of the 33 potential druggable genes. The results suggested that most identified genes and AD likely share a causal variant within the region (figure 2 and online supplemental table S11). For three prior genes, EPHX2 and SIGLEC11 produced significant results in both datasets (PPH\(_3\) >0.8), and SERPINB1 also reached significant levels in the blood (PPH\(_3\) +PPH\(_4\) =0.98) but not in the brain (PPH\(_4\) +PPH\(_5\) =0.42) in the further colocalisation analysis. When only the GWAS of clinically diagnosed AD was used, SIGLEC11 and SERPINB1 passed FDR of <0.05 in the brain eQTL, and the FDR of SIGLEC11 in the blood was very close to 0.05 (online supplemental table S12).

Additionally, during our replication, MR using whole blood and brain frontal lobe cis-eQTL from GTEx V8,\(^21\) SIGLEC11, SERPINB1, LIMK2, CD46 and ADAM10 showed suggestive significance (p<0.05) in either the blood or brain (online supplemental table S13 and S14).

### Association between AD druggable genes and markers

Previous reviews were screened to identify the biochemical and neuroimaging markers of AD.\(^1\) Owing to the restricted GWAS data on AD markers, we only found the following markers with sufficient data: cerebrospinal fluid (CSF) A\(_\beta\),\(^32\) CSF p-tau,\(^32\) circulating t-tau,\(^33\) total/right/left hippocampal volume,\(^34,\) \(^35\) cortical surface and thickness,\(^36\) white matter hyperintensities,\(^35\) and white matter integrity (fractional anisotropy and mean diffusivity).\(^37\)

We found that blood and brain EPHX2 may be associated with the whole hippocampal volume (unadjusted p values=0.019 and 0.0088, respectively; online supplemental table S15). However, no association was identified between three prior druggable genes (EPHX2, SERPINB1 and SIGLEC11) and the aforementioned AD markers. In addition, using the marker GWAS as an exposure and AD as the outcome, we observed that only the whole brain surface affected the AD outcome (unadjusted p value=0.00869, online supplemental table S16).

### Phenome-wide MR analysis of AD prior druggable genes

Because most drugs function through blood circulation, we assessed whether the three AD-associated expressions in the blood had beneficial or deleterious effects on other indications. Therefore, we performed a broader MR screening of 783 non-AD diseases or traits in the UK Biobank (SAIGE V.0.29, online supplemental table S17).\(^1\) Overall, no significant association was identified (FDR <0.05) using the IVW method (figure 3), although we observed some trends. Higher blood EPHX2 levels may potentially benefit hypertensive chronic kidney disease (OR 0.73, p=0.00027) (figure 3 and online supplemental table S18). In addition, high levels of SERPINB1 caused pituitary gland disorders and hypothalamic control (OR 1.67, p=0.0023) (figure 3 and online supplemental table S19). Blood SIGLEC11 levels were most likely associated with periapical abscesses (OR 0.74, p=0.0025) (figure 3 and online supplemental table S20). No other diseases may be linked to these druggable genes that...
Cognition passed a significant FDR (0.05), and the summary results are presented in online supplemental table S18–20.

DISCUSSION
The development of novel therapeutic agents for AD is extremely challenging. A major cause for this dilemma is the unknown pathophysiology of AD. In this study, based on blood and brain druggable eQTLs, we found 33 druggable gene expressions that may influence AD outcomes, 5 of which were also found in a repeated study. Indeed, our study provides robust evidence that three genes (EPHX2, SERPINB1 and SIGLEC11) are causally associated with AD, and all show evidence of genetic colocalisation with AD outcomes. Furthermore, we found associations between EPHX2 and whole hippocampal volume, which may provide potential clues to the pathological mechanisms involved in AD. In addition, phenome-wide MR highlighted additional beneficial indications of therapeutics targeting the three AD-associated genes and indicated a few potential safety concerns.

EPHX2 encodes a member of the epoxide hydrolase family, which is found in the cytosol and peroxisomes. It binds to specific epoxides and converts them to the corresponding dihydrodiols. Soluble epoxide hydrolase (sEH) expression is elevated in the hippocampus of AD mice, and sEH levels are also higher in the brain of patients with AD compared with healthy individuals. In pharmacological studies, the genetic deletion of EPHX2 or the downregulation of sEH level reduces Aβ deposition in the brain of patients with AD compared with healthy individuals. Furthermore, a proteome-wide association study integrating a smaller AD GWAS with the discovery of the Religious Orders Study, Memory and Ageing Project brain proteomes and a non-druggable genomic MR study using protein quantitative trait locus data reported that EPHX2 might affect AD. Consistent with these results, we provide druggable genetic evidence for the directionally consistent effects of EPHX2 in the blood or brain tissue on AD outcomes. We also observed that higher levels of EPHX2 may be associated with lower hippocampal volume. More importantly, through phenome-wide MR analysis, we observed no significant side effects associated with EPHX2 expression (online supplemental table S18). SEH expression is elevated in the AD hippocampus, whether the pathological accumulation of sEH accelerates hippocampal atrophy and leads to AD requires further research. PF-750, a drug that inhibits fatty acid amide hydrolase, weakly inhibits sEH. However, this compound has not yet reached phase I clinical trials (preclinical/research compound). Another drug, AR9281, may also interact with EPHX2 without a clear direction yet. Overall, our results indicated that EPHX2 is a promising target for treating AD by affecting hippocampal volume. However, PF-750 or compounds that target EPHX2 deserve further investigation.

Serpin family B member 1 (SERPINB1) encodes a member of the serpin family of proteinase inhibitors, maintaining homeostasis by neutralising overexpressed proteinase activity through their function as suicide substrates. A locus associated with Aβ42 within SERPINB1 on chromosome 6p25 has been reported to be associated with AD risk in a GWAS study. A previous study indicated that the intronic SERPINB1 variant rs316341 affects the expression of SERPINB1 in various tissues, including the hippocampus, suggesting that SERPINB1 may influence AD through an Aβ-associated mechanism. In addition, SERPINB1 is upregulated in patients with AD, and higher levels of SERPINB1 expression in the prefrontal cortex are associated with higher levels of amyloidosis. Our MR study also revealed that higher SERPINB1 levels increased the risk of AD. However, we found no association between SERPINB1 and AD biomarkers using MR in this study. Because of the unavailability of Figure 3
Manhattan plot for phenome-wide MR results of blood EPHX2, SERPINB1 and SIGLEC11. Note: ordinate representation of the p value in phenome-wide MR results. A dot represents a disease trait, and different colours represent the MR result of different expressions. EPHX2, epoxide hydrolase 2; MR, Mendelian randomisation.
of a large proportion of AD pathological marker GWAS data, negative results are not credible. More basic experiments and larger GWAS data are needed to determine the relationship between SERPINB1 and Aβ or other AD biomarkers. SERPINB1 is the only druggable gene under the tier 3 B term (encoding secreted or extracellular proteins, proteins with more distant similarity to approved drug targets and members of key drug-gene families not already included in tier 1 or 2) according to Finan et al.13 Currently, there are no drugs with SERPINB1 as a clear target. Heparin from bovines is predicted to interact with SERPINB1 (www.novoseek.com); however, its exact role is unclear. Our phenome-wide MR analysis showed that the association of the drug with blood SERPINB1 produced no side effects in other systems. Hence, drug development targeting SERPINB1 may be promising, and further research is needed to elucidate the mechanism of action of SERPINB1 on the risk of AD.

SIGLEC11, another robust AD-associated gene identified in the present study, encodes a member of the sialic acid-binding immunoglobulin-like lectin family. It is mainly expressed in peripheral blood leukocytes, macrophages and microglia and binds to an α-2-8-linked sialic acid.49 It is a risk factor for AD.40 SIGLEC11 may be associated with ceramide-related inflammation and anti-inflammatory pathways in AD.50 In addition to our findings, higher levels of SIGLEC11 may trigger an inflammatory response that can produce neurotoxic effects or promote the deposition of pathological proteins in AD, although the exact mechanism is unknown. Further investigation of SIGLEC11 in the context of AD is required.

In addition, the remaining expression products could provide further insights into anti-AD drug discovery. When comparing the MR results using eQTLs derived from the two cohorts, SIGLEC11, SERPINB1, LIMK2, CD46 and ADAM10 were repeated in at least one tissue sample. LIMK2 regulates cortical development by regulating neural progenitor cell proliferation and migration.31 Consistent with our findings, a previous study identified CD46 as significantly dysregulated in late-onset AD.52 Moreover, ADAM10 is an important enzyme in AD pathology and may function in AD via astrocytes.33, 34 2,4-Diacetylphloroglucinol reduces Aβ production and secretion by regulating ADAM10 and its intracellular trafficking in cellular and animal models of AD.34 Furthermore, the development of ADAM10 endocytosis inhibitors for AD treatment yielded promising preclinical results.35 Therefore, multiple studies support the drug targets identified in our study.

Since our primary objective was to provide genetic evidence to improve success rates in clinical trials for AD drug discovery, we decided to reduce the number of false positives. Therefore, we integrated two-stage MR (discovery and validation) in blood and brain tissues and used colocalisation analysis to identify robust druggable genes. We removed SNPs directly related to and brain tissues and used colocalisation analysis to identify we decided to reduce the number of false positives. Therefore, 55 eQTLs for AD treatment yielded promising preclinical results.52 ADAM10 is a risk factor for AD.50 The strengths of our study include the following. First, the number of IVs, we only ran heterogeneity and pleiotropy analyses for MR between AD markers and AD outcomes but not for the MR-involved eQTLs. Second, because the data were locked, many AD markers were not included in our study, such as neurofibrillary tangles, inflammatory plaques, soluble TREM2 and positron emission tomography images, leading to potential associations being missed. Third, the findings were not repeated at the protein level, considering that many genes did not have IVs after filtering the p value when we conducted a preliminary study. In addition, our initial findings were based on AD GWAS, including clinical and proxy cases. MR with proxy cases yields counterintuitive findings.55 Nonetheless, SIGLEC11 and SERPINB1 reached adjusted significance only in clinically diagnosed AD GWAS. This may be due to insufficient sample size and the fact that EPHX2 was not repeated. Furthermore, it is difficult to determine the most appropriate tissue for AD discovery. The genes that reached significant levels in the blood and brain tissues may have stronger evidence. Another key limitation is that MR analysis cannot fully recapitulate clinical trials. MR analysis differs from clinical trials, which typically investigate comparably high drug doses over a short time.51 Additionally, the GWASs conducted in the UK Biobank were restricted to Caucasians, which might restrict the applicability of our findings to other groups and races. Moreover, although all participants in the UK Biobank were of European ancestry, geographical clustering was not adjusted in the original GWASs. It was reported that the health outcomes appear geographically structured and that coincident structure in health outcomes and genotype data can yield biased associations in UK Biobank.57 Therefore, our results might be biased by population heterogeneity in the UK Biobank.58 Future studies with participants from the same region were worthy to be performed. Furthermore, we only explored the side effects of EPHX2, SIGLEC11 and SERPINB1 eQTL in diseases from the UK Biobank. However, the effects of drugs on their targets are quite extensive, and many off-target effects cannot be explored using MR. Further exploration is required through subsequent basic and clinical trials to gain a more comprehensive understanding. Finally, there were not as many approved drugs as expected, making it difficult to study new uses of the old drugs; however, we found three robust druggable genes.

CONCLUSION
This study found three robust druggable genes (EPHX2, SERPINB1 and SIGLEC11) and 30 candidate druggable genes for AD. In addition, AD biomarkers and phenotype-wide MR analyses of EPHX2, SERPINB1 and SIGLEC11 were conducted. In summary, we provide genetic evidence supporting the potential therapeutic benefits of three druggable genes for AD, which will be useful for prioritising AD drug development.

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Contributors W-M: conception, methodology, analysis and writing of the manuscript; J-XG: methodology, analysis and writing; MD, Q-DO, ZJ, K-FY and W-CC: validation; BC and YW: validation and supervision of the project; Y-PC: wrote, revised, discussed the final edition and responsible for the overall content; all authors read and approved the final version of the manuscript.

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