Causal effect of insulin resistance on small vessel stroke and Alzheimer's disease: A Mendelian randomization analysis

Mengyuan Zhou | Hao Li | Yongjun Wang | Yuesong Pan | Yilong Wang

Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, Beijing, China

Correspondence
Yuesong Pan and Yilong Wang, Department of Neurology, Beijing Tiantan Hospital, Capital Medical University, No.119 South 4th Ring West Road, Fengtai District, Beijing 100070, China. Emails: yuesongpan@ncrcnd.org.cn (YP); yilong528@aliyun.com (YW)

Funding information
The study is supported by grants from the National Natural Science Foundation of China (No. 81971091, 81825007), the Beijing Outstanding Young Scientist Program (No. BJJWZJH01201910025030), the Beijing Hospitals Authority Youth Program (QML20190501), the Youth Beijing Scholar Program (No. 010), the Beijing Talent Project - Class A: Innovation and Development (No. 2018A12), the "National Ten-Thousand Talent Plan", Leadership of Scientific and Technological Innovation, and the National Key R&D Program of China (No. 2017YFC1307900, 2017YFC1307905)

Abstract
Background and purpose: The causal effect of insulin resistance on small vessel stroke and Alzheimer’s disease (AD) was controversial in previous studies. We therefore applied Mendelian randomization (MR) analyses to identify the causal effect of insulin resistance on small vessel stroke and AD.

Methods: We selected 12 single-nucleotide polymorphisms (SNPs) associated with fasting insulin levels and five SNPs associated with "gold standard" measures of insulin resistance as instrumental variables in MR analyses. Summary statistical data on SNP–small vessel stroke and on SNP–AD associations were derived from studies by the Multi-ancestry Genome-Wide Association Study of Stroke consortium (MEGASTROKE) and the Psychiatric Genomics Consortium-Alzheimer Disease Workgroup (PGC-ALZ) in individuals of European ancestry. Two-sample MR estimates were conducted with inverse-variance-weighted, robust inverse-variance-weighted, simple median, weighted median, weighted mode-based estimator, and MR pleiotropy residual sum and outlier (MR-PRESSO) methods.

Results: Genetically predicted higher insulin resistance had a higher odds ratio (OR) of small vessel stroke (OR 1.23, 95% confidence interval [CI] 1.05–1.44, p = 0.01 using fasting insulin; OR 1.25, 95% CI 1.07–1.46, p = 0.006 using gold standard measures of insulin resistance) and AD (OR 1.13, 95% CI 1.04–1.23, p = 0.004 using fasting insulin; OR 1.02, 95% CI 1.00–1.03, p = 0.03 using gold standard measures of insulin resistance) using the inverse-variance-weighted method. No evidence of pleiotropy was found using MR-Egger regression.

Conclusion: Our findings provide genetic support for a potential causal effect of insulin resistance on small vessel stroke and AD.

Keywords
Alzheimer's disease, causality, insulin resistance, Mendelian randomization, small vessel stroke

INTRODUCTION
Cerebral small vascular disease (CSVD) is a common age-related pathological process that affects perforating vessels and surrounding tissue of the brain and insidiously leads to white-matter hyperintensities, lacunar infarcts and microbleeds, which may be associated with cognitive impairment, dementia, gait disturbances and mood changes [1,2]. Alzheimer’s disease (AD) is the most prevalent neurodegenerative disease evolving to dementia, while CSVD accounts for approximately 45% of dementia cases [3]. Increasing evidence suggests there are overlapping risk factors for CSVD and AD, such as hypertension and abnormal glucose metabolism [4,5]. As AD and CSVD are both geriatric comorbidities, concurrence of AD and CSVD pathology is commonly observed in patients with AD [6].
Insulin resistance is a pathological condition resulting from decreased insulin sensitivity both in the periphery and brain [7]. It has been demonstrated that insulin resistance is associated with the risk of ischemic stroke and poor outcomes of ischemic stroke [8–10]. Recent observational studies have reported that insulin resistance is associated with an increased risk of CSVD [11–14]. Moreover, brain insulin resistance was recently demonstrated to play an important role in AD [15]. However, the causal effect of insulin resistance on small vessel stroke and AD was controversial in previous Mendelian randomization (MR) analyses [16,17].

Mendelian randomization is an analytic technique, simulating the design of randomized controlled trials, that uses genetic variants associated with exposure as instrumental variables to infer causality between such exposure and risk of diseases [18]. Because genetic variants are randomly allocated at meiosis and independent of many other confounders, MR analysis can avoid potential biases of conventional observational studies and reverse causality. In the present study, we aimed to determine the causal associations of insulin resistance with the risk of small vessel stroke and AD based on MR analysis.

MATERIALS AND METHODS

Study Design and Data Sources

We performed this MR analysis in accordance with the recommendations of the STROBE-MR statement [19]. A two-sample MR analysis was applied to evaluate the causal effect of insulin resistance on the risk of small vessel stroke and AD (Figure 1). MR design is based on the theory that genotypes are randomly assorted at meiosis and independent of confounding factors, so that potential confounders and reverse causation can be controlled for and more reliable causal inferences can be obtained [18]. MR relies on three assumptions: (i) the instrument is associated with the exposure; (ii) the instrument influences the outcome only through the exposure; and (iii) the instrument is not associated with other confounders.

Single nucleotide polymorphisms (SNPs) that are associated with insulin resistance and satisfy the MR assumptions were extracted as instrumental variables. SNPs for insulin resistance based on fasting insulin were obtained from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC) [20]. SNPs for insulin resistance based on “gold standard” measures were obtained from the GENeticS of Insulin Sensitivity consortium (GENESIS) [21]. Data on the association of SNPs with small vessel disease were obtained from the Multi-ancestry Genome-Wide Association Study of Stroke consortium (MEGASTROKE) [22]. Data on the association of SNPs with AD were obtained from the Psychiatric Genomics Consortium-Alzheimer Disease Workgroup (PGC-ALZ) [23]. All genome-wide association studies (GWASs) of these consortia are based on individuals of European ancestry. The characteristics of the GWASs used in the present study are shown in Table S1. All the original studies had obtained ethical approval, and all participants had provided informed consent.

Selection of genetic variants

We used two methods to measure insulin resistance: (i) a common proxy of insulin resistance measured by fasting insulin and (ii) gold standard measures of insulin resistance based on the euglycemic-hyperinsulinemic clamp and the insulin suppression test. We selected SNPs associated with insulin resistance from two GWAS consortia: MAGIC [20] and GENESIS [21]. The MAGIC assessed potential SNPs associated with fasting insulin in 108,557 non-diabetic individuals of European ancestry [20]. The consortium using the Illumina CardioMetabochip containing ~66,000 SNPs for a range of cardiovascular and metabolic traits, contributed ~1000 SNPs for fasting insulin. The estimates of associations were adjusted for body mass index and fasting insulin was natural logarithm transformed. The consortium identified 12 loci that achieved significance (p < 5 × 10^{-8}) for insulin resistance. The F statistic of these index variants was 1016, indicating sufficient strength of the instruments. We extracted independent genetic variants without linkage disequilibrium with other SNPs for insulin resistance. The GENESIS analyzed GWAS data on 2764 individuals and replication in 2860 individuals of European ancestry with direct, standard measures of insulin resistance from four cohort GWASs (the RISC consortium, the Uppsala

FIGURE 1 Conceptual framework for the Mendelian randomization analysis of insulin resistance and risk of small vessel stroke and Alzheimer’s disease (AD). The design is based on the assumption that the genetic variants are associated with insulin resistance, but not with confounders, and affect small vessel stroke and AD only through insulin resistance. SNP, single-nucleotide polymorphism
Longitudinal Study of Adult Men, the EUGENE2 consortium, and the Stanford Insulin Suppression Test [21]. Since no SNPs reached GWAS significance levels of \(p < 5 \times 10^{-8}\) in the initial meta-analysis, the investigators took forward variants representing four of the top signals into follow-up studies, from which they identified that five SNPs most strongly associated with insulin resistance reached significance levels of \(p < 6 \times 10^{-6}\). The \(F\) statistic of these index variants was 72, indicating sufficient strength of the instruments. The associations of the 17 individual SNPs with insulin resistance are shown in Table 1. These insulin resistance-associated SNPs were at different loci and there was no linkage disequilibrium \(\left(r^2 < 0.2\right)\). Furthermore, they were not associated with other potential risk factors related to CSVD or AD at a genome-wide significance level \(\left(p < 5 \times 10^{-5}\right)\) after performing a search in the PhenoScanner database [24].

### Outcomes

Summary statistics for the associations of individual SNPs with small vessel stroke, defined as stroke caused by small vessel disease, were acquired from previously published GWASs conducted by the MEGASTROKE consortium [22]. The MEGASTROKE consortium is a multi-ancestry genome-wide association meta-analysis of stroke and stroke subtypes that tests ~8 million SNPs and indels with minor allele frequency \(\geq 0.01\) in 521,612 individuals (67,162 stroke cases and 454,450 controls) for association with stroke. Analysis in European individuals involved 40,585 stroke cases and 406,111 controls.

Summary statistics for the associations of individual SNPs with AD were acquired from the previously published GWASs of the PGC-ALZ [23]. The PGC-ALZ study is a three-phase genome-wide meta-analysis involving 455,258 individuals (71,880 cases and 383,378 controls) of European ancestry. The genome-wide meta-analysis of clinically diagnosed AD case–control status was based on four independent consortia: the PGC-ALZ, the International Genomics of Alzheimer’s Project (IGAP), the Alzheimer’s Disease Sequencing Project (ADSP), and the UK Biobank. Diagnosis of AD in PGC-ALZ was made according to the recommendations of the National Institute on Aging-Alzheimer’s Association, the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) and the International Classification of Diseases Tenth Revision (ICD-10) research criteria. AD in the IGAP and ADSP is autopsy-confirmed or clinically diagnosed according to the NINCDS-ADRDA criteria, the Diagnostic and Statistical Manual of Mental Disorders (DSM) Fourth Edition criteria, the Alzheimer’s Disease Diagnostic and Treatment Centers State of California criteria, or the DSM Third Edition Revised criteria. The UK Biobank constructs an AD-by-proxy status as part of a self-report questionnaire based on parental AD information weighted by age during the in-person interview, which is additionally confirmed with ICD-10 codes (G30, F00) in medical records. All individuals were entered into a meta-analysis of clinical AD GWASs and the AD-by-proxy GWAS. The associations of individual SNPs for insulin resistance with small vessel stroke and AD are shown in Table 2.

### Statistics analysis

We performed two-sample MR analyses on summary statistics to evaluate the impact of insulin resistance-associated genetic variants on small vessel stroke and AD. The SNP–insulin resistance and SNP–outcome associations were used to calculate estimates of insulin resistance–outcome (small vessel stroke and AD) associations using inverse-variance-weighted (IVW) MR analysis. In sensitivity analyses, we performed complementary analyses using robust IVW, MR-Egger, simple median, weighted median, weighted mode-based estimator (MBE), and MR pleiotropy residual sum and outlier (MR-PRESSO) methods. These methods include pleiotropic or invalid instruments that are more robust to potential violations of the standard instrumental variable assumption. The MR-Egger method can identify and control for bias due to directional pleiotropy (SNPs influence the outcome through different biological pathways other than exposure) [25]. The weighted median method allows stronger SNPs to contribute more toward the estimate and, therefore, allows them to contribute more toward stronger causal inferences [26]. The MR-PRESSO method was applied to identify and correct potential outliers in multi-instrument summary-level MR testing [27]. Potential pleiotropic effects of these SNPs were evaluated via MR-Egger regression, in which the slope represents causal estimates corrected by pleiotropy and the intercept represents the average pleiotropic effects of all SNPs. Heterogeneity of SNPs was estimated using the Cochran Q statistic. If there is heterogeneity, random-effects IVW models are applied; otherwise, the fixed-effect IVW model is applied. To estimate the influence of outlying or pleiotropic genetic variants, we conducted a leave-one-out analysis in which we re-estimated the effect by sequentially dropping one SNP at a time.

The odds ratios (ORs) with their 95% confidence intervals (CIs) per one standard deviation log-transformed genetically predicted increase in insulin resistance were used to represent the association between insulin resistance and outcomes (small vessel stroke and AD). We additionally plotted the association of each genetic variant with insulin resistance against its effect on the outcomes. The Bonferroni-adjusted significance for small vessel stroke and AD was calculated as \(p < 0.025 \left(\frac{0.05}{2} = 0.025\right)\) to ensure the validity of our conclusions. In the present analyses, two-sided \(p\) values \(< 0.05\) were taken to indicate statistical significance for a potential, yet to be confirmed, causal association, and a two-sided \(p\) value \(< 0.025\) was taken to indicate statistical significance for a causal association. All analyses were conducted using R 3.5.3 (R Development Core Team).

### RESULTS

#### Causal association of insulin resistance with small vessel stroke

The IVW MR analyses showed positive correlations between insulin resistance and the risk of small vessel stroke (OR 1.23, 95% CI 1.05–1.44, \(p = 0.01\) using fasting insulin; OR 1.25, 95% CI 1.07–1.46,
EFFECT OF INSULIN RESISTANCE ON SMALL VESSEL STROKE AND ALZHEIMER’S DISEASE

EFFECT OF INSULIN RESISTANCE ON SMALL VESSEL STROKE AND ALZHEIMER’S DISEASE

Figure 2). Associations between each instrumental variable with insulin resistance and the risk of small vessel stroke are shown in Figure 4a,b.

In sensitivity analyses, significant causal associations were observed for the risk of small vessel stroke using the robust IVW, simple median and weighted median methods with insulin resistance assessed by fasting insulin and gold standard measures (all \( p < 0.025 \); Figure 2). Potential significant causal associations were observed between insulin resistance assessed by fasting insulin, and risk of small vessel stroke using weighted MBE and MR-PRESSO methods (both \( p < 0.05 \); Figure 2). No significant causal association was observed between insulin resistance assessed by gold standard measures and small vessel stroke using weighted MBE and MR-PRESSO methods.

Mendelian randomization-Egger regression showed no evidence of heterogeneity for the associations of insulin resistance with small vessel stroke (\( p = 0.53 \) using fasting insulin; \( p = 0.44 \) using gold standard measures). The result of leave-one-out sensitivity analyses indicated that the association between insulin resistance and small vessel stroke was not substantially affected by any individual SNP (data shown in Figures S1 and S2).

**Causal association of insulin resistance with AD**

The IVW method showed a positive causal association between insulin resistance, assessed by fasting insulin, and the risk of AD (OR 1.13, 95% CI 1.04–1.23, \( p = 0.004 \); Figure 3) and a potential causal association of higher insulin resistance, assessed by gold standard measures, with AD (OR 1.02, 95% CI 1.00–1.03; \( p = 0.03 \); Figure 3). Associations between each instrumental variable with insulin resistance and risk of AD are shown in Figure 4c,d.

**Table 1** Characteristics of included single-nucleotide polymorphisms associated with insulin resistance

| Exposure                  | SNPs                  | Prioritized genes | Position (hg19)  | EA/OA | EAF   | Association with exposure |
|---------------------------|-----------------------|-------------------|------------------|-------|-------|---------------------------|
| FI                        | rs2943645             | IRS1              | chr2:226807424   | T/C   | 0.63  | \( 2.26 \times 10^{-19} \) |
| FI                        | rs10195252            | GRB14             | chr2:165221337   | T/C   | 0.60  | \( 1.26 \times 10^{-16} \) |
| FI                        | rs2126259             | PPP1R3B           | chr8:9222556     | T/C   | 0.11  | \( 3.30 \times 10^{-13} \) |
| FI                        | rs4865796             | ARL5              | chr5:53308421    | A/G   | 0.67  | \( 2.16 \times 10^{-12} \) |
| FI                        | rs17036328            | PPARG             | chr3:12365484    | T/C   | 0.86  | \( 3.59 \times 10^{-12} \) |
| FI                        | rs731839              | PEPPD             | chr19:38590905   | G/A   | 0.34  | \( 5.13 \times 10^{-12} \) |
| FI                        | rs974801              | TET2              | chr4:106290513   | G/A   | 0.38  | \( 3.27 \times 10^{-11} \) |
| FI                        | rs459193              | ANKRDS5/MAP3K1    | chr5:55842508    | G/A   | 0.73  | \( 1.15 \times 10^{-10} \) |
| FI                        | rs6822892             | PDGFC             | chr4:157954125   | A/G   | 0.68  | \( 2.58 \times 10^{-10} \) |
| FI                        | rs4846565             | LYPAL1            | chr1:217788727   | G/A   | 0.67  | \( 1.76 \times 10^{-9} \)  |
| FI                        | rs3822072             | FAM13A1           | chr4:89960292    | A/G   | 0.48  | \( 1.80 \times 10^{-8} \)  |
| FI                        | rs6912327             | UHRF1BP1          | chr6:34872900    | T/C   | 0.80  | \( 2.26 \times 10^{-8} \)  |
| IR by gold standard measures | rs9877159             | -                 | chr3:190699342   | G/A   | 0.90  | \( 5.56 \times 10^{-6} \)  |
| IR by gold standard measures | rs117421960           | TMEM64            | chr8:91723406    | T/G   | 0.96  | \( 3.56 \times 10^{-6} \)  |
| IR by gold standard measures | rs1801280             | NAT2              | chr8:18257854    | T/C   | 0.55  | \( 3.74 \times 10^{-6} \)  |
| IR by gold standard measures | rs1208                | NAT2              | chr8:18258316    | A/G   | 0.57  | \( 9.81 \times 10^{-7} \)  |
| IR by gold standard measures | rs1775921             | RNU6-270P         | chr10:29045858   | T/C   | 0.91  | \( 4.33 \times 10^{-6} \)  |

Note: Genomic coordinates refer to human genome build 37 (hg19).

Abbreviations: EA, effect allele; EAF, effect allele frequency; FI, fasting insulin; IR, insulin resistance; OA, other allele; SE, standard error; SNP, single-nucleotide polymorphism.
resistance assessed by gold standard measures and AD using robust IVW method (OR 1.02, 95% CI 1.00–1.03; \( p = 0.02 \); Figure 3). However, no significant association was found for the risk of AD using simple median, weighted median, and weighted MBE methods with insulin resistance assessed by fasting insulin or gold standard measures.

Mendelian randomization-Egger regression showed no evidence of heterogeneity for the associations of insulin resistance with AD (\( p = 0.03 \) for fasting insulin; \( p = 0.32 \) for gold standard measures).

The result of leave-one-out sensitivity analyses indicated that the association between insulin resistance and AD was not affected by any individual SNP (data shown in Figures S3 and S4).

**DISCUSSION**

In the present study, genetically predicted insulin resistance, either based on fasting insulin or gold standard measures (including euglycemic-hyperinsulinemic clamp and insulin suppression test), showed potential causal associations with increased risk of small vessel stroke and AD. Findings on the causal association between insulin resistance and small vessel stroke were more robust in sensitivity analyses using different instruments and statistical models. A previous study showed that the frequency of vascular risk factors differed among subtypes of ischemic stroke [28]. Insulin resistance is widely considered a core feature of metabolic disorders but is not limited to type 2 diabetes mellitus, and it was found to be an independent risk factor for small vessel stroke and a predictor of its severity in Korean individuals [12]. Previous studies have used different indices of insulin resistance, such as homeostasis model assessment-estimated insulin resistance (HOMA-IR) index and insulin resistance score, to demonstrate that insulin resistance is associated with occurrence of lacune, although the results of the different indices are not identical [11,12]. The HOMA-IR and triglyceride-glucose index, calculated as proxies of insulin resistance, were also indicated to be risk factors for increased CSVD burden [13,14]. However, previous MR studies on the relationship between insulin resistance and small vessel stroke, where insulin resistance was assessed by a proxy measure (fasting insulin), did not yield significant results [29,30], and the GWAS summary data on small vessel stroke

---

**TABLE 2** Genetic association of insulin resistance related genetic variants with small vessel stroke and Alzheimer’s disease

| SNPs      | EA/OA | small vessel stroke | Alzheimer’s disease |
|-----------|-------|---------------------|---------------------|
|           |       | Beta    | SE    | p value | Beta    | SE    | p value |
| FI        | rs2943645 | T/C | 0.0188 | 0.0234 | 0.4213 | 0.0048 | 0.0022 | 0.0268 |
| FI        | rs10195252 | T/C | 0.0386 | 0.0232 | 0.0962 | 0.0015 | 0.0021 | 0.4899 |
| FI        | rs2126259 | T/C | −0.0078 | 0.0397 | 0.8440 | 0.0027 | 0.0035 | 0.4355 |
| FI        | rs4865796 | A/G | 0.0338 | 0.0247 | 0.1719 | −0.0008 | 0.0023 | 0.7367 |
| FI        | rs17036328 | T/C | 0.0326 | 0.0343 | 0.3428 | 0.0052 | 0.0033 | 0.1137 |
| FI        | rs731839 | G/A | 0.0344 | 0.0250 | 0.1697 | 0.0017 | 0.0022 | 0.4568 |
| FI        | rs974801 | G/A | −0.0117 | 0.0236 | 0.6181 | 0.0011 | 0.0022 | 0.6206 |
| FI        | rs459193 | G/A | 0.0299 | 0.0272 | 0.2724 | 0.0053 | 0.0024 | 0.0259 |
| FI        | rs6822892 | A/G | −0.0221 | 0.0248 | 0.3728 | 0.0006 | 0.0022 | 0.8027 |
| FI        | rs4846565 | G/A | −0.0046 | 0.0241 | 0.8480 | −0.0013 | 0.0023 | 0.5552 |
| FI        | rs3822072 | A/G | 0.0306 | 0.0227 | 0.1774 | −0.0013 | 0.0021 | 0.5285 |
| FI        | rs6912327 | T/C | 0.0372 | 0.0289 | 0.1984 | 0.0044 | 0.0025 | 0.0774 |
| IR by gold standard measures | rs9877159 | G/A | 0.0602 | 0.0417 | 0.1484 | 0.0076 | 0.0033 | 0.0203 |
| IR by gold standard measures | rs117421960 | T/G | 0.0039 | 0.0673 | 0.9543 | 0.0072 | 0.0060 | 0.2328 |
| IR by gold standard measures | rs1801280 | T/C | 0.0498 | 0.0226 | 0.0278 | 0.0020 | 0.0021 | 0.3496 |
| IR by gold standard measures | rs1208 | A/G | 0.0406 | 0.0239 | 0.0899 | 0.0009 | 0.0021 | 0.6704 |
| IR by gold standard measures | rs1775921 | T/C | 0.0201 | 0.0393 | 0.6083 | −0.0004 | 0.0035 | 0.9126 |

Abbreviations: EA, effect allele; FI, fasting insulin; IR, insulin resistance; OA, other allele; SE, standard error; SNP, single-nucleotide polymorphism.
were not the most recent and comprehensive. Our MR analysis evaluated insulin resistance using both fasting insulin and gold standard measures, and summary statistics for the association of individual SNPs with small vessel stroke were acquired from the recent large-scale GWAS-MEGASTROKE consortium.

A previous observational study indicated that insulin resistance was associated with cognitive impairment in elderly patients with primary hypertension [31]. Radiographic studies taking advantage of positron emission tomography found that higher insulin resistance was associated with regional cortical hypometabolism in the frontal,
parietotemporal, and cingulate regions (which are vulnerable to AD pathology) in patients who were cognitively normal but complicated with prediabetes or type 2 diabetes mellitus or had a parental history of AD [32,33]. In a post-mortem study, insulin resistance was found to be associated with β-amyloid plaques [34]. However, the evidence of a genetic effect of insulin resistance on AD was conflicting in previous MR studies [16,17]. The MR analysis performed by Walter et al. [17] suggested that insulin sensitivity assessed by subscores formed from a subset of type 2 diabetes mellitus-associated SNPs affects the risk of AD. Nevertheless, the SNPs classified as related to insulin sensitivity were not valid instrumental variables, which may lead to violation of assumptions. Ostergaard et al. [16] performed an MR study and found no association between insulin resistance and AD. We used data from a much larger and more recent consortium and assessed insulin resistance not only through fasting insulin but also through gold standard measures, thus providing a more reliable causal effect of insulin resistance on the risk of AD.

There are several potential mechanisms involved in insulin resistance and CSVD and cognitive impairment. First, futile response of adipocytes to the actions of insulin is observed in patients with insulin resistance, which can lead to lipolysis and dyslipidemia and increase the risk of atherosclerosis [35]. Second, subclinical inflammation and oxidative stress are common in patients with insulin resistance, which can result in endothelium impairment and increased blood–brain barrier permeability, eventually contributing to initial onset and subsequent progression of CSVD [13,14].

FIGURE 4 Associations of insulin resistance related variants with risk of small vessel stroke and Alzheimer disease (AD). (a) Genetic association of fasting insulin-related single-nucleotide polymorphisms (SNPs) and small vessel stroke. (b) Genetic association of standardly measured insulin resistance-related SNPs and small vessel stroke. (c) Genetic association of fasting insulin-related SNPs and AD. (d) Genetic association of standardly measured insulin resistance-related SNPs and AD. Red line indicates the estimate of effect using the inverse-variance weighted method. Circles indicate marginal genetic associations with insulin resistance and risk of outcome for each variant. Error bars indicate 95% confidence intervals. SD, standard deviation [Colour figure can be viewed at wileyonlinelibrary.com]
Third, insulin was demonstrated to increase cerebral perfusion, which can be impaired owing to insulin resistance and subsequently lead to neuronal dysfunction [36]. Fourth, accumulation of β-amyloid and hyperphosphorylation of tau protein are core features of AD [37]. It was reported that insulin can accelerate β-amyloid clearance from the brain and prevent extracellular deposition as well as fibril and plaque formation in normal conditions [38]. Finally, insulin and insulin resistance are suggested to implicate in the aggregation of tau protein. Dysfunction of insulin can lead to tau hyperphosphorylation of specific amino acids such as Ser and Thr [39].

A major strength of the present study is the MR design. In our MR analysis we used genetic variants to assess the causal effect of exposure (insulin resistance) for disease (small vessel stroke and AD) based on multiple insulin resistance-related SNPs and effects of SNP on outcomes from GWASs. An MR study can avoid reverse causality and minimize confounding by environmental factors. Results from MR studies can reflect life-long exposure as genetic variants are allocated at meiosis. Second, we examined the causal effect of insulin resistance on outcomes based on data of GWASs with large sample sizes (5386 small vessel stroke cases and 406,111 controls, 71,880 AD and 383,378 controls). Third, our study selected a more common proxy of insulin resistance measured by fasting insulin and gold standard measures of insulin resistance based on the euglycemic-hyperinsulinemic clamp and the insulin suppression test. The consistent results of two definitions of insulin resistance and different MR methods in sensitivity analyses make our conclusion more convincing. Additionally, as cognitive impairment is a clinical manifestation of CSVD, similar results for small vessel stroke and AD may indicate the role of combined vascular pathology and AD in dementia.

We acknowledge several limitations of this study. The main limitation of the MR study is that pleiotropy could possibly affect the reliability of the results. As biased causal effect estimates may exist, it is often difficult to exclude potential confounders. In the present study, no pleiotropic effects were observed in the MR-Egger regression analysis. Additionally, we note that the association found between genetically predicted insulin resistance with small vessel stroke using the MR-Egger method is in the opposite direction to that observed using other MR methods, but is not statistically significant. The MR-Egger method is sensitive to outliers, whereas the weighted median and MBE are robust to outliers, and MR-PRESSO removes outliers, improving the consistency with the estimates obtained using the IVW method. As the p values for some sensitivity analyses did not reach statistical significance, the results should be interpreted with caution. Second, the five SNPs used as instrumental variables for insulin resistance by gold standard measures did not reach the p level of $5 \times 10^{-8}$ due to both the limited size of the sample in which the original GWAS data were obtained and other limitations[21]. Therefore, caution is needed in interpreting the causal relationships between insulin resistance assessed by gold standard measures and small vessel stroke and AD. In addition, our MR analysis was based on individuals of European ancestry, which may limit the generalizability of our findings to other ethnicities.

In conclusion, the present results provide genetic support for a potential causal effect of insulin resistance on small vessel stroke and AD. It is widely known that both type 1 and type 2 diabetes mellitus share long-term microvascular injury/dysfunction, but previous studies have focused more on the microvasculature of eyes and kidneys. The results of the present study suggest that insulin resistance might contribute to cerebral small vessel injury. Further investigations are needed to verify the association and the mechanism involved.

ACKNOWLEDGMENTS
We thank the MAGIC and GENESIS consortia for providing insulin resistance-related SNPs. Data on small vessel stroke were provided by the MEGASTROKE (PubMed ID: 29531354) investigators. Data on AD were provided by the PGC-ALZ consortium (PubMed ID: 30617256).

CONFLICT OF INTEREST
All authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS
Mengyuan Zhou: Conceptualization (lead); Data curation (equal); Formal analysis (lead); Investigation (lead); Methodology (equal); Writing – original draft (lead). Hao Li: Project administration (supporting); Resources (supporting); Supervision (supporting). Yongjun Wang: Project administration (supporting); Resources (supporting); Supervision (supporting). Yuesong Pan: Conceptualization (lead); Data curation (lead); Formal analysis (equal); Funding acquisition (equal); Methodology (lead); Project administration (equal); Writing – review and editing (lead). Yilong Wang: Data curation (equal); Formal analysis (equal); Funding acquisition (equal); Project administration (equal); Supervision (equal); Writing – review and editing (equal).

DATA AVAILABILITY STATEMENT
Data of the present study are publicly available and may also be available from the corresponding authors upon request.

ORCID
Mengyuan Zhou https://orcid.org/0000-0002-2078-0767
Yongjun Wang https://orcid.org/0000-0002-9976-2341
Yilong Wang https://orcid.org/0000-0002-3267-0039

REFERENCES
1. Pantoni L. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. Lancet Neurol. 2010;9:689-701.
2. Debette S, Schilling S, Duperron MG, Larsson SC, Markus HS. Clinical significance of magnetic resonance imaging markers of vascular brain injury: a systematic review and meta-analysis. JAMA Neurol. 2019;76:81-94.
3. Gorelick PB, Scuteri A, Black SE, et al. Vascular contributions to cognitive impairment and dementia: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2011;42:2672-2713.
4. Gardener H, Wright CB, Rundek T, Sacco RL. Brain health and shared risk factors for dementia and stroke. Nat Rev Neurol. 2015;11:651-657.
5. Cannistraro RJ, Badi M, Eidelman BH, Dickson DW, Middlebrooks EH, Meschia JF. CNS small vessel disease: a clinical review. *Neurology*. 2019;92:1146-1156.

6. Attems J, Jellinger KA. The overlap between vascular disease and Alzheimer’s disease—lessons from pathology. *BMC Med*. 2014;12:206.

7. Gray SM, Meijer RI, Barrett EJ. Insulin regulates brain function, but how does it get there? *Diabetes*. 2014;63:3992-3997.

8. Rundek T, Gardener H, Xu Q, et al. Insulin resistance and risk of ischemic stroke among nondiabetic individuals from the northern Manhattan stroke study. *Arch Neurol*. 2010;67:1195-1200.

9. Thacker EL, Psaty BM, McKnight B, et al. Fasting and post-glucose load measures of insulin resistance and risk of ischemic stroke in older adults. *Stroke*. 2011;42:3347-3351.

10. Pan Y, Jing J, Chen W, et al. Post-glucose load measures of insulin resistance and prognosis of nondiabetic patients with ischemic stroke. *J Am Heart Assoc*. 2017;6:e004990.

11. Dearborn JL, Schneider AL, Sharrett AR, et al. Obesity, insulin resistance, and incident small vessel disease on magnetic resonance imaging: atherosclerosis risk in communities study. *Stroke*. 2015;46:3131-3136.

12. Lee JE, Shin DW, Yun JM, et al. Insulin resistance is a risk factor for silent lacunar infarction. *Stroke*. 2016;47:2938-2944.

13. Yang X, Zhang S, Dong Z, et al. Insulin resistance is a risk factor for overall cerebral small vessel disease burden in old nondiabetic healthy adult population. *Front Aging Neurosci*. 2019;11:127.

14. Nam KW, Kwon HM, Jeong HY, Park JH, Kwon H, Jeong SM. High triglyceride-glucose index is associated with subcortical cerebral small vessel disease in a healthy population: a cross-sectional study. *Cardiovasc Diabetol*. 2020;19:53.

15. de la Monte SM. Insulin resistance and neurodegeneration: progress towards the development of new therapeutics for Alzheimer’s disease. *Drugs*. 2017;77:47-65.

16. Ostergaard SD, Mukherjee S, Sharp SJ, et al. Associations between potentially modifiable risk factors and Alzheimer disease: a Mendelian randomization study. *PloS Med*. 2015;12:e1001841. discussion e.

17. Walter S, Marden JR, Kuzbansky LD, et al. diabetic phenotypes and late-life dementia risk: a mechanism-specific Mendelian randomization study. *Alzheimer Dis Assoc Disord*. 2016;30:15-20.

18. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey SG. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133-1163.

19. Skrivankova VW, Richmond RC, Woolf BAR, et al. Strengthening the reporting of observational studies in epidemiology using Mendelian randomization: the STROBE-MR statement. *JAMA*. 2021;326:1614-1621.

20. Scott RA, Lagou V, Welch RP, et al. Large-scale association analyses identify new loci influencing glycemic traits and provide insight into the underlying biological pathways. *Nat Genet*. 2012;44:991-1005.

21. Knowles JW, Xie W, Zhang Z, et al. Identification and validation of N-acetyltransferase 2 as an insulin sensitivity gene. *J Clin Invest*. 2015;125:1739-1751.

22. Malik R, Chauhan G, Traylor M, et al. Multiancestry genome-wide association study of 520,000 subjects identifies 32 loci associated with stroke and stroke subtypes. *Nat Genet*. 2018;50:524-537.

23. Jansen IE, Savage JE, Watanabe K, et al. Genome-wide meta-analysis identifies new loci and functional pathways influencing Alzheimer’s disease risk. *Nat Genet*. 2019;51:404-413.

24. Staley JR, Blackshaw J, Kamat MA, et al. PhenoScanner: a database of human genotype-phenotype associations. *Bioinformatics*. 2016;32:3207-3209.

25. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. *Int J Epidemiol*. 2015;44:512-525.

26. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304-314.

27. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50:693-698.

28. Schulz UG, Rothwell PM. Differences in vascular risk factors between etiological subtypes of ischemic stroke: importance of population-based studies. *Stroke*. 2003;34:2050-2059.

29. Liu J, Rutten-Jacobs L, Liu M, Markus HS, Traylor M. Causal impact of type 2 diabetes mellitus on cerebral small vessel disease: a Mendelian randomization analysis. *Stroke*. 2018;49:1325-1331.

30. Larsson SC, Scott RA, Traylor M, et al. Type 2 diabetes, glucose, insulin, BMI, and ischemic stroke subtypes: Mendelian randomization study. *Neurology*. 2017;89:454-460.

31. Ma L, Feng M, Qian Y, et al. Insulin resistance is an important risk factor for cognitive impairment in elderly patients with primary hypertension. *Yonsei Med J*. 2015;56:89-94.

32. Baker LD, Cross DJ, Minoshima S, Belongia D, Watson GS, Craft S. Insulin resistance and Alzheimer-like reductions in regional cerebral glucose metabolism for cognitively normal adults with prediabetes or early type 2 diabetes. *Arch Neurol*. 2011;68:51-57.

33. Willette AA, Bendlin BB, Starks EJ, et al. Association of insulin resistance with cerebral glucose uptake in late-middle-aged adults at risk for Alzheimer disease. *JAMA Neurol*. 2015;72:1013-1020.

34. Talbot K, Wang HY, Kazi H, et al. Demonstrated brain insulin resistance in Alzheimer’s disease patients is associated with IGF-1 resistance, IRS-1 dysregulation, and cognitive decline. *J Clin Invest*. 2012;122:1316-1338.

35. Eckel RH, Grundy SM, Zimet PZ. The metabolic syndrome. *Lancet*. 2005;365:1415-1428.

36. Fu Z, Wu J, Nesil T, Li MD, Aylor KW, Liu Z. Long-term high-fat diet induces hippocampal microvascular insulin resistance and cognitive dysfunction. *Am J Physiol Endocrinol Metab*. 2017;312:E89-E97.

37. Bloom GS. Amyloid-beta and tau: the trigger and bullet in Alzheimer’s disease pathogenesis. *JAMA Neurol*. 2014;71:505-508.

38. Watson GS, Peskind ER, Asthana S, et al. Insulin increases CSF Abeta42 levels in normal older adults. *Neurology*. 2003;60:1899-1903.

39. Rad SK, Arya A, Karimian H, et al. Mechanism involved in insulin resistance via accumulation of beta-amyloid and neurofibillary tangles: link between type 2 diabetes and Alzheimer’s disease. *Drug Des Devel Ther*. 2018;12:3999-4021.

**SUPPORTING INFORMATION**

Additional supporting information may be found in the online version of the article at the publisher’s website.

How to cite this article: Zhou M, Li H, Wang Y, Pan Y, Wang Y. Causal effect of insulin resistance on small vessel stroke and Alzheimer’s disease: A Mendelian randomization analysis. *Eur J Neurol*. 2022;29:698-706. doi:10.1111/ene.15190