Causal effect of Insulin Resistance on Small Vessel Stroke and Alzheimer's Disease: A Mendelian Randomization Analysis

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Abstract

Background

The causal effect of insulin resistance on small vessel stroke and Alzheimer Disease was controversial in previous studies.

Methods

We selected 12 single-nucleotide polymorphisms (SNPs) associated with body mass index (BMI)-adjusted fasting insulin levels and 5 SNPs associated with gold standard measures of insulin resistance as instrumental variables in Mendelian randomization (MR) analyses. Summary statistical data of SNP-small vessel stroke and of SNP-AD associations were derived from the Multi-ancestry Genome-Wide Association Study of Stroke Consortium and Psychiatric Genomics Consortium-Alzheimer's Disease Workgroup data of 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 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 BMI-adjusted fasting insulin; OR 1.25; 95% CI 1.07–1.46; *P* = 0.006 using gold standard measure of insulin resistance) and AD (OR 1.13; 95% CI 1.04–1.23; *P* = 0.004 using BMI-adjusted 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 causal effect of insulin resistance on small vessel stroke and AD. Further investigation on the involved mechanisms is needed.

Introduction

Cerebral small vascular disease (CSVD) is an age-related pathologic process affecting small arteries, arterioles, venules and capillaries of the brain, of which the prevalence ranging from 5% for people aged 50 years to almost 100% for people aged 90 years.[1, 2] With shared potential risk factors such as abnormal glucose metabolism,[3, 4] CSVD accounts for ~ 45% of dementia cases.[5] Dementia is reported to occur in 50 million people worldwide in 2018 and suppose to triple to 152 million by 2050.[6] The two related diseases, CSVD and Alzheimer Disease, are two common disorders in elderly people.

Insulin resistance is a pathological condition resulting from decreased insulin sensitivity both in peripheral and in the brain.[7] It has been well demonstrated that insulin resistance is associated with a risk and poor outcomes of ischemic stroke.[8–10] Recently, observational studies have reported that insulin resistance was associated with an increased risk of CSVD.[11–14] Moreover, brain insulin resistance was recently demonstrated to play an important role in Alzheimer Disease.[15] However, the causal effect of insulin resistance on small vessel stroke and Alzheimer Disease was controversial in previous Mendelian randomization (MR) analyses.[16, 17]

MR 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 Alzheimer Disease based on MR analysis.

Methods

Study Design and Data Sources

We applied a 2-sample MR analysis to evaluate the causal effect of insulin resistance on the risk of small vessel stroke and Alzheimer Disease (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 and more reliable causal inferences can be obtained.[18] MR relies on three assumptions: 1) the instrument is associated with the exposure; 2) the instrument influences the outcome only through the exposure; 3) the instrument is not associated with other confounders.

Single nucleotide polymorphisms (SNPs) that are associated with insulin resistance and satisfy with the MR assumptions were extracted as instrumental variable. SNPs for insulin resistance based on fasting insulin were obtained from the Meta-Analyses of Glucose and Insulin-related traits Consortium (MAGIC).[19] SNPs for insulin resistance based on gold standard measures were obtained from the GENEticS of Insulin Sensitivity consortium (GENESIS).[20] Data on association of SNPs with small vessel disease were obtained from the Multiancestry Genome-Wide Association Study of Stroke consortium (MEGASTROKE).[21] Data on association of SNPs with Alzheimer's Disease were obtained from the Psychiatric Genomics Consortium-Alzheimer Disease Workgroup (PGC-ALZ).[22] All the consortia are based upon genome-wide association studies (GWASs) of individual of European ancestry. Characteristics of the GWAS studies used in this study are presented in Table 1.

Selection of Genetic Variants

We used two methods to measure insulin resistance: 1) a common proxy of insulin resistance measured by fasting insulin, and 2) 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: the MAGIC[19] and the GENESIS.[20] The MAGIC assessed potential SNPs associated with fasting insulin in 108,557 non-diabetic individuals of European ancestry.[19] The consortia using the Illumina CardioMetabochip containing ~66000 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 (BMI) and the fasting insulin was natural logarithm transformed. They identified 12 loci that achieved significance ($P < 5\times10^{-8}$) for the insulin resistance. We extracted independent genetic variants without linkage disequilibrium with other SNPs for insulin resistance. The GENSIS analyzed GWAS data of 2764 individual and replication in 2860 individuals of European ancestry with direct, standard measures of insulin resistance from four cohort GWASs (the RISC consortium, Uppsala Longitudinal Study of Adult Men, the EUGENE2 consortium, and the Stanford Insulin Suppression Test[20]). They identified 5 SNPs that reached significance ($P < 6\times10^{-6}$) for the insulin resistance. The association of the 17 individual SNPs with the insulin resistance is presented in Table 2. These insulin resistance-associated SNPs were at different loci and without any linkage disequilibrium ($r^2 < 0.2$). Furthermore, they were not associated with other potential risk factors related to CSVD or Alzheimer Disease at a genome-wide significance level ($P < 5\times10^{-8}$) after performing a search in the PhenoScanner database.[23]

Outcomes

Summary statistics for the associations of individual SNP with small vessel stroke were acquired from previously published GWAS of Multiancestry Genome-Wide Association Study of Stroke (MEGASTROKE) consortium.[21] The MEGASTROKE consortium is a multi-ancestry genome-wide association meta-analysis on stroke and stroke subtypes that tests ~8 million SNPs and indels with minor allele frequency ≥ 0.01 in 521,612 individuals (67,162 stoke cases and 454,450 controls) for association with stroke. Analysis in European individuals involved 40,585 stoke cases and 406,111 controls (Methods in the online-only Data Supplement).

Summary statistics for the associations of individual SNPs with Alzheimer Disease were acquired from the previously published GWAS of the Psychiatric Genomics Consortium-Alzheimer's Disease Workgroup (PGC-ALZ).[22] The PGC-ALZ is a three-pahase 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 Alzheimer Disease case–control status was based on four independent consortia: the PGC-ALZ, the International Genomics of Alzheimer's Project (IGAP), Alzheimer's Disease Sequencing Project (ADSP), and UK Biobank. The diagnosis of Alzheimer Disease in PGC-ALZ is in accordance with the recommendations of National Institute on Aging-Alzheimer's Association, National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) and International Classification of Diseases Tenth Revision (ICD-10) research criteria. Alzheimer Disease in the IGAP and ADSP is autopsy-confirmed or clinically diagnosed with NINCDS-ADRDA criteria, Diagnostic and Statistical Manual of Mental Disorders (DSM) Fourth Edition) criteria, Alzheimer's Disease Diagnostic and Treatment Centers State of California criteria, or DSM Third Edition Revised criteria. UK Biobank constructs an Alzheimer Disease-by-proxy status as part of self-report questionnaire based on parental Alzheimer Disease 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 Alzheimer Disease GWAS and the Alzheimer Disease-by-proxy GWAS (Methods in the online-only Data Supplement). The associations of individual SNPs for insulin resistance with small vessel stroke and Alzheimer Disease are presented in Table 3.

Statistics Analysis

We performed 2-sample MR analyses on summary statistics to evaluate the impact of insulin resistance-associated genetic variants on small vessel stroke and Alzheimer Disease. The SNP-insulin resistance and SNP-outcome associations were used to calculate estimates of insulin resistance-outcome (small vessel stroke and Alzheimer Disease) associations using inverse-variance weighted (IVW) MR analysis. In sensitivity analyses, we complementarily performed 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).[24] The weighted median method allows stronger SNPs to contribute more toward the estimate and, therefore, allows them to contribute more toward stronger causal inferences.[25] The MR-PRESSO method was applied to identify and correct potential outliers in multi-instrument summary-level MR testing.[26] Potential pleiotropic effects of these SNPs were evaluated via the 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 by Cochran Q statistic. If there is heterogeneity, random-effects IVW models are applied; otherwise, the fixed-effect IVW model is applied. In order 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 1 SNP at a time.

The odds ratios (ORs) with their 95% confidence interval (CI) per 1-SD log-transformed genetically predicted increase in insulin resistance were used to represent the association between insulin resistance and outcomes (small vessel stroke and Alzheimer Disease). 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 Alzheimer Disease was calculated as $P < 0.025$ (0.05/2 = 0.025) to ensure the validity of our conclusions. In the present analyses, 2-sided $P < 0.05$ was considered statistically significant for a potential, but yet to be confirmed, causal association, and a 2-sided $P < 0.025$ was considered to be statistically significant for a causal association. All analyses were conducted with R 3.5.3 (R Development Core Team).

Results

Causal Association of Insulin Resistance with Small Vessel Stroke

The IVW MR analyses showed significant associations between higher insulin resistance and the risk of small vessel stroke (OR 1.23; 95% CI 1.05-1.44; *P* = 0.01 using BMI-adjusted fasting insulin; OR 1.25; 95% CI 1.07-1.46; *P* = 0.006 using gold standard measures of insulin resistance; Fig. 2). Associations between each instrumental variant with insulin resistance and the risk of small vessel stroke are displayed in Fig. 4 (A and 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 BMI-adjusted fasting insulin and gold standard measures (all *P* < 0.025; Fig. 2). Potential significant causal associations were observed between insulin resistance assessed by BMI-adjusted fasting insulin, and risk of small vessel stroke using weighted MBE and MR-PRESSO methods (both *P* < 0.05; Fig. 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.

MR-Egger regression showed no evidence of directional pleiotropy for the associations of insulin resistance with small vessel stroke (*P* = 0.97 using BMI-adjusted fasting insulin; *P* = 0.09 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 not shown).

Causal Association of Insulin Resistance with Alzheimer Disease

The IVW method showed a significant causal association of higher insulin resistance assessed by BMI-adjusted fasting insulin with an increased risk of Alzheimer Disease (OR 1.13; 95% CI 1.04-1.23; *P* = 0.004; Fig. 3) and a potential causal association of higher insulin resistance assessed by gold standard measures with Alzheimer Disease (OR 1.02; 95% CI 1.00-1.03; *P* = 0.03; Fig. 3). Associations between each instrumental variant with insulin resistance and risk of Alzheimer Disease are displayed in Fig. 4 (C and D).

In sensitivity analyses, significant causal associations were found between insulin resistance (OR 1.13; 95% CI 1.04-1.22; *P* = 0.003 using BMI-adjusted fasting insulin; OR 1.02; 95% CI 1.00-1.03; *P* = 0.02 using gold standard measures; Fig. 3) and Alzheimer Disease. In addition, a potentially significant association was observed between insulin resistance assessed by BMI-adjusted fasting insulin and risk of Alzheimer Disease using MR-Egger (*P* = 0.03; Fig. 3), and a significant association was observed using MR-PRESSO methods (*P* = 0.01; Fig. 3). No significant association was found for the risk of Alzheimer Disease using simple median, weighted median, and weighted MBE methods with insulin resistance assessed by BMI-adjusted fasting insulin and gold standard measures.

MR-Egger regression showed no evidence of directional pleiotropy for the associations of insulin resistance with Alzheimer Disease (*P* = 0.08 using fasting insulin adjusted by BMI; *P* = 0.78 using gold standard measures). The result of leave-one-out sensitivity analyses indicated that the association between insulin resistance and Alzheimer Disease was not affected by any individual SNP (Data not shown).

Discussion

In the present study, genetically predicted insulin resistance, no matter based on a surrogate measure (BMI-adjusted fasting insulin) or gold standard measures (including euglycemic-hyperinsulinemic clamp and insulin suppression test), was causally associated with an increased risk of small vessel stroke and Alzheimer Disease. The finding was robust in sensitivity analyses with different instruments and statistical models.

A previous study showed that the frequency of vascular risk factors differed among subtypes of ischemic stroke.[27] Insulin resistance is widely considered a core feature of metabolic disorders but not exclusive to type 2 diabetes mellitus (T2DM), it was found to be an independent risk factor for small vessel stroke and predictor of its severity in Koreans.[12] Insulin resistance measured by homeostasis model assessment–estimated insulin resistance (HOMA-IR) index was reported to be positively correlated with silent lacunar stroke,[12] although another study found that the insulin resistance score rather than

HOMA-IR or insulin was associated with incident lacunes.[11] HOMA-IR and the triglyceride-glucose index, calculated as proxies of insulin resistance, were recently indicated as risk factors of increased CSVD burden.[13, 14] However, previous MR studies on the relationship between insulin resistance and small vessel stroke, in which insulin resistance was evaluated through a proxy measure (fasting insulin), have yielded insignificant results,[28, 29], and the GWAS summary data of small vessel stroke was published several years ago. In our MR analysis, we evaluated insulin resistance using both BMI-adjusted fasting insulin and gold standard measures, and summary statistics for the association of individual SNPs with small vessel stroke was acquired from the large-scale GWAS-MEGASTROKE consortium.

A previous observational study indicated that insulin resistance was associated with cognitive impairment in elderly patients with primary hypertension.[30] Radiographic studies taking the advantage of positron emission tomography found that higher insulin resistance was associated with reginal cortical hypometabolism in frontal, parietotemporal, and cingulate regions (which are vulnerable to Alzheimer Disease pathology) in patients who were cognitively normal but complicated with prediabetes or T2DM or had a parental history of Alzheimer Disease.[31, 32] In a post-mortem study, insulin resistance was found to associate with β-amyloid plaques.[33] However, the evidence of a genetic effect of insulin resistance on Alzheimer Disease was conflicting in previous MR studies.[16, 17] The MR analysis performed by Stefan and colleagues showed that insulin sensitivity assessed by subscores formed from a subset of T2DM-associated SNPs was implicated to affect Alzheimer Disease risk.[17] Nevertheless, the SNPs classified as related to insulin sensitivity were not qualified instrument variable, which may lead to violation of assumptions. Østergaard et al published an MR study and found no association between insulin resistance and Alzheimer Disease.[16] We used data from a much larger and more recent consortia and assessed insulin resistance not only through a proxy but also through gold standard measures, thus providing a more reliable causal effect of insulin resistance on the risk of Alzheimer Disease.

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.[34] 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] Third, insulin was demonstrated to increase cerebral perfusion, which can be impaired owing to insulin resistance and subsequently lead to neuronal dysfunction.[35] Fourth, accumulation of β-amyloid and hyperphosphorylation of tau protein are core features of Alzheimer Disease.[36] 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 condition.[37] Finally, insulin and insulin resistance have been implicated in the of tau protein aggregation. Dysfunction of insulin can lead to tau hyperphosphorylation of specific amino acid such as Ser and Thr.[38]

A major strength of the present study is the MR design. Our MR analysis used genetic variants to assess the causal effect of exposure (insulin resistance) for disease (small vessel stroke and Alzheimer disease), 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 406111 controls; 71880 Alzheimer disease and 383378 controls). Third, previous MR studies often used proxies of insulin resistance such as fasting insulin and subscore formed from a subset of T2DM risk loci,[17, 29] whereas our study selected a 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 between two definitions of insulin resistance and different MR methods in sensitivity analyses make our conclusion more convincible. Additionally, as cognitive impairment is a clinical manifestation of CSVD, similar results for small vessel stroke and Alzheimer Disease further validated our findings.

We acknowledge several limitations to our work. A main limitation of the MR study is that pleiotropy is a potential threat to the reliability of the results. As biased causal effect estimates may exist, it is often challenging to rule out potential confounders.

However, our main findings of insulin resistance and small vessel stroke and Alzheimer Disease were consistent in sensitivity analyses, and no pleiotropic effects was observed in MR-Egger regression analysis. Further, our MR analysis was based on individuals of European ancestry, which may limit the generalizability of our findings to other ethnicities.

Conclusions

Our results provide genetic support for causal effect of insulin resistance on small vessel stroke and Alzheimer Disease. Association of insulin resistance and ischemic stroke is well-documented. Both type 1 diabetes mellitus and T2DM share long-term microvascular injury/dysfunction, however, previous studies are more focusing on microvasculature of eyes and kidneys. The present study suggested that insulin resistance might contributed to cerebral small vessel injury. Further investigation on the involved mechanisms is needed.

Abbreviations

BMI: body mass index; CI: confidence interval; CSVD: cerebral small vascular disease; GWAS: genome-wide association study; HOMA-IR: homeostasis model assessment–estimated insulin resistance; HR: hazard ratio; IVW: inverse-variance weighted; MBE: mode-based estimator; MR: Mendelian randomization; MR-PRESSO: Mendelian randomization pleiotropy residual sum and outlier; OR: odds ratio; SNPs: single nucleotide polymorphisms; T2DM: type 2 diabetes mellitus.

Declarations

Acknowledgments

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Author contributions

MZ, YP, and YLW contributed to the conception and design of the study; MZ and YP contributed to the analysis of data, preparing the figures, and drafting the text; YP, YLW, YJW and HL contributed to manuscript revision.

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Availability of data and materials

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

Ethics approval and consent to participate

All of the original studies had received ethical approvals, and all participants had provided informed consent.

Consent for publication

The manuscript was approved by all authors for publication.

Competing interests

All authors declare that they have no competing interests.

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Tables

Table 1 Characteristics of the GWAS studies used in this study

Phenotype	Consortium	N	Ethnicity	Genotype data	PMID
Exposure (insulin resistance) *					
Fasting insulin adjusted for BMI	MAGIC	Up to 103,489 individuals	European	GWAS array and metabochip array	22885924
Insulin sensitivity by gold standard measures	GENESIS	2,764 individuals	European	GWAS array	25798622
Outcomes					
Small vessel stroke	MEGASTROKE	5,386/406,111	European	GWAS array	29531354
Alzheimer's disease	PGC-ALZ	71,880/ 383,378	European	GWAS array	30617256

GWAS genome-wide association study, *MAGIC* the Meta-Analyses of Glucose and Insulin-related traits Consortium, *GENESIS* the GENEticS of Insulin Sensitivity Consortium, *MEGASTROKE* the Multi-ancestry Genome-Wide Association Study of Stroke Consortium, *PGC-ALZ* the Psychiatric Genomics Consortium-Alzheimer's Disease Workgroup.

Table 2 Characteristics of included SNPs associated with insulin resistance

	Beta	SE	P value					
Exposure	SNPs	Prioritized genes	Position (hg19)	EA/OA	EAF	Association with exposure		
FI-adjBMI	rs2943645	*IRS1*	chr2:226807424	T/C	0.63	0.0193	0.0021	2.26×10^{-19}
FI-adjBMI	rs10195252	*GRB14*	chr2:165221337	T/C	0.60	0.0174	0.0021	1.26×10^{-16}
FI-adjBMI	rs2126259	*PPP1R3B*	chr8:9222556	T/C	0.11	0.0238	0.0033	3.30×10^{-13}
FI-adjBMI	rs4865796	*ARL15*	chr5:53308421	A/G	0.67	0.0154	0.0022	2.16×10^{-12}
FI-adjBMI	rs17036328	*PPARG*	chr3:12365484	T/C	0.86	0.0212	0.0030	3.59×10^{-12}
FI-adjBMI	rs731839	*PEPD*	chr19:38590905	G/A	0.34	0.0148	0.0021	5.13×10^{-12}
FI-adjBMI	rs974801	*TET2*	chr4:106290513	G/A	0.38	0.0139	0.0021	3.27×10^{-11}
FI-adjBMI	rs459193	*ANKRD55/MAP3K1*	chr5:55842508	G/A	0.73	0.0147	0.0023	1.15×10^{-10}
FI-adjBMI	rs6822892	*PDGFC*	chr4:157954125	A/G	0.68	0.0138	0.0022	2.58×10^{-10}
FI-adjBMI	rs4846565	*LYPLAL1*	chr1:217788727	G/A	0.67	0.0132	0.0022	1.76×10^{-9}
FI-adjBMI	rs3822072	*FAM13A1*	chr4:89960292	A/G	0.48	0.0116	0.0021	1.80×10^{-8}
FI-adjBMI	rs6912327	*UHRF1BP1*	chr6:34872900	T/C	0.80	0.0165	0.0029	2.26×10^{-8}
IR by gold standard measures	rs9877159	-	chr3:190699342	G/A	0.90	0.21	0.05	5.56×10^{-6}
IR by gold standard measures	rs117421960	*TMEM64*	chr8:91723406	T/G	0.96	0.4	0.09	3.56×10^{-6}
IR by gold standard measures	rs1801280	*NAT2*	chr8:18257854	T/C	0.55	0.13	0.03	3.74×10^{-6}
IR by gold standard measures	rs1208	*NAT2*	chr8:18258316	A/G	0.57	0.13	0.03	9.81×10^{-7}
IR by gold standard measures	rs1775921	*RNU6-270P*	chr10:29045858	T/C	0.91	0.23	0.05	4.33×10^{-6}

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

SNP single-nucleotide polymorphism, *EA* effect allele, *OA* other allele, *EAF* effect allele frequency, *SE* standard error, *FI* fasting insulin, *BMI* body mass index, *IR* insulin resistance.

Table 3 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-adjBMI	rs2943645	T/C	0.0188	0.0234	0.4213	0.0048	0.0022	0.0268
FI-adjBMI	rs10195252	T/C	0.0386	0.0232	0.0962	0.0015	0.0021	0.4899
FI-adjBMI	rs2126259	T/C	-0.0078	0.0397	0.8440	0.0027	0.0035	0.4355
FI-adjBMI	rs4865796	A/G	0.0338	0.0247	0.1719	-0.0008	0.0023	0.7367
FI-adjBMI	rs17036328	T/C	0.0326	0.0343	0.3428	0.0052	0.0033	0.1137
FI-adjBMI	rs731839	G/A	0.0344	0.0250	0.1697	0.0017	0.0022	0.4568
FI-adjBMI	rs974801	G/A	-0.0117	0.0236	0.6181	0.0011	0.0022	0.6206
FI-adjBMI	rs459193	G/A	0.0299	0.0272	0.2724	0.0053	0.0024	0.0259
FI-adjBMI	rs6822892	A/G	-0.0221	0.0248	0.3728	0.0006	0.0022	0.8027
FI-adjBMI	rs4846565	G/A	-0.0046	0.0241	0.8480	-0.0013	0.0023	0.5552
FI-adjBMI	rs3822072	A/G	0.0306	0.0227	0.1774	-0.0013	0.0021	0.5285
FI-adjBMI	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

SNP single-nucleotide polymorphism, *EA* effect allele, *OA* other allele, *SE* standard error, *FI* fasting insulin, *BMI* body mass index, *IR* insulin resistance.

Figures

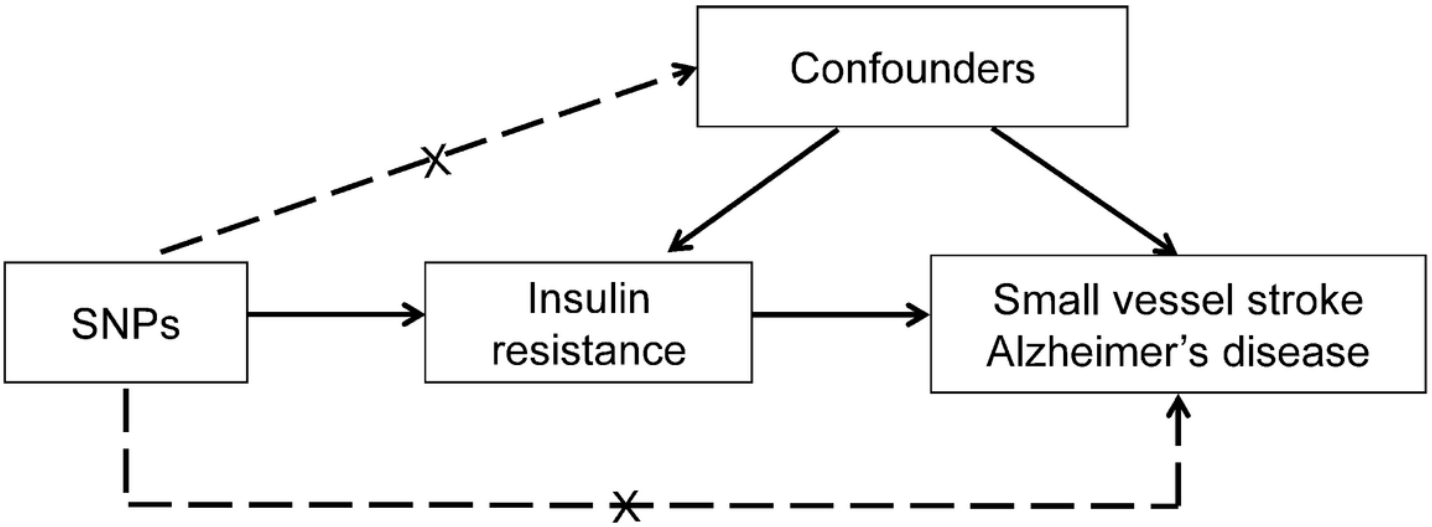

Figure 1

Conceptual framework for the Mendelian randomization analysis of insulin resistance and risk of small vessel stroke and Alzheimer disease. The design is based on the assumption that the genetic variants are associated with insulin resistance, yet with confounders, and influence the small vessel stroke and Alzheimer disease only through insulin resistance.

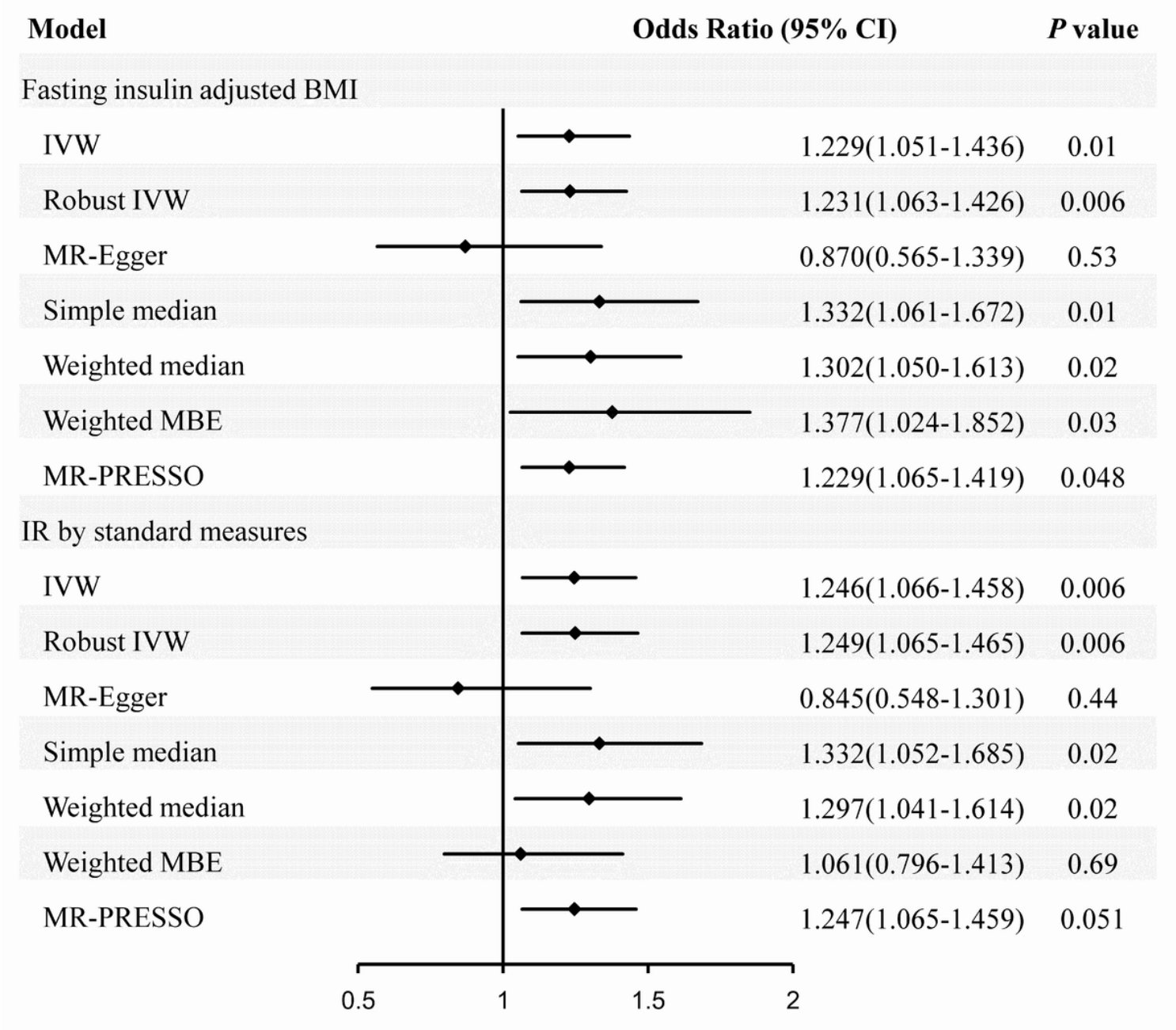

Figure 2

Causal effect estimates of genetically predicted insulin resistance on small vessel stroke. Estimates are derived from inverse-variance weighted (IVM) method of Mendelian randomization analysis and represented as odds ratio (95% CI). Sensitivity analysis included robust IVW, MR-Egger, simple median, weighted median, weighted Mode-Based Estimator (MBE), and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods. BMI indicated body mass index.

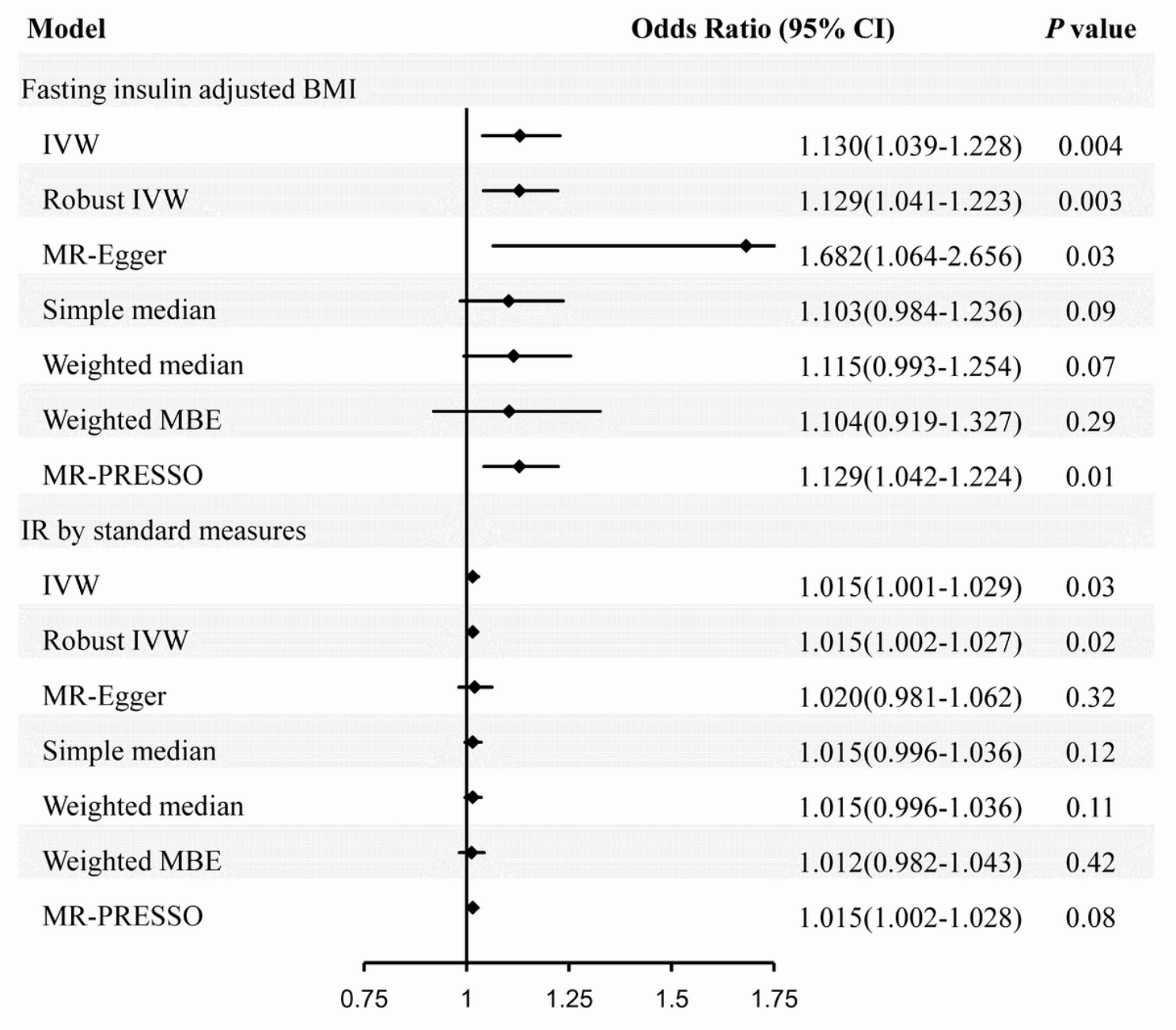

Figure 3

Causal effect estimates of genetically predicted insulin resistance on Alzheimer's disease. Estimates are derived from inverse-variance weighted (IVM) method of Mendelian randomization analysis and represented as odds ratio (95% CI). Sensitivity analysis included robust IVW, MR-Egger, simple median, weighted median, weighted Mode-Based Estimator (MBE), and MR Pleiotropy Residual Sum and Outlier (MR-PRESSO) methods. BMI indicated body mass index.

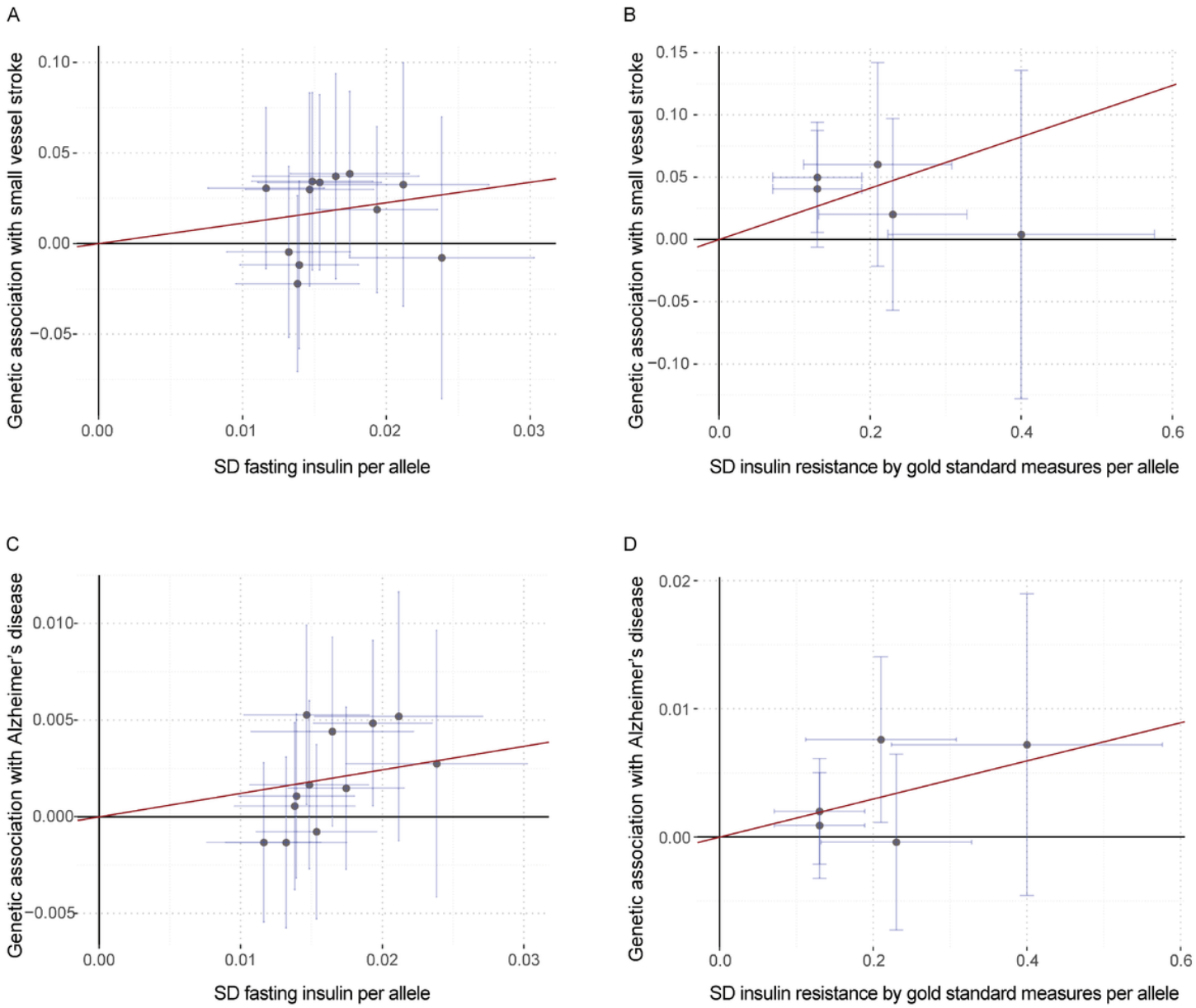

Figure 4

Associations of insulin resistance related variants with risk of small vessel stroke and Alzheimer disease. (A) Genetic association of fasting insulin-related 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 Alzheimer's Disease. (D) Genetic association of standardly measured insulin resistance-related SNPs and Alzheimer's Disease. The red line indicates the estimate of effect using IVW method. Circles indicate marginal genetic associations with insulin resistance and risk of outcome for each variant. Error bars indicate 95% confidence intervals. SD indicates standard error; IVW indicates inverse-variance weighted.