Causal association of plasminogen activators and their inhibitors with Alzheimer's disease: a Mendelian randomization study

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Abstract

Introduction: Alzheimer's disease (AD) is the most common cause of dementia and contributes to a huge burden of disease worldwide. Observational studies have found that tissue plasminogen activator (t-PA) inhibits the development of AD, but little is known about urokinase plasminogen activator (u-PA) or plasminogen activator inhibitor-1 (PAI-1). At present, the causal relationship is not clear. Therefore, this study intended to explore the relationship between plasminogen activators and their inhibitors with Alzheimer's disease through the Mendelian randomization method, so as to provide a reference for the prevention and control of Alzheimer's disease.
Material and methods: To investigate causal pathways, we conducted a two-sample Mendelian randomization study using pooled statistics from genome-wide association studies. Inverse-variance weighted (IVW), Mendelian randomization-Egger (MR-Egger), weighted-median, Mendelian randomization pleiotropy residual sum and outlier (MR-PRESSO) and Mendelian randomization-robust adjusted profile score (MR-RAPS) methods were used to evaluate the robustness of the results.
Results: In the outcome of AD (more controls excluded), the IVW effect of PAI-1 OR (95% CI) was found as follows: 1.543 (1.010–2.356), whose interval does not include 1 and $p = 0.0448$, which suggested that PAI-1 was positively correlated with the risk of AD (more controls excluded). The IVW model, weighted median, MR-PRESSO and MR-RAPS all showed similar results (all odds ratios [ORs] > 1), and the two outcomes were consistent.
Conclusions: Our results showed that gene-predicted PAI-1 in Mendelian stochastic analysis was associated with an increased risk of AD.

Key words: Mendelian randomization, tissue plasminogen activator, urokinase type plasminogen, plasminogen activator inhibitor 1, Alzheimer's disease.

Introduction

Alzheimer's disease (AD) is the most common cause of dementia, and contributes to a significant disease burden worldwide [1, 2]. Aggregation and deposition of amyloid-β (Aβ) is an important pathological mechanism of AD [3].

The role of the plasminogen system in the pathogenesis of AD is closely related to Aβ. Animal experimental studies demonstrated that tissue plasminogen activator (t-PA)-mediated reduction of plasminase activity could lead to Aβ deposition during aging [4, 5], and t-PA administration could reduce AD-related pathological progression by reducing the Aβ level in the brain and improving cognitive function [6], which may be related to t-PA's involvement in Aβ clearance and neurovascular bonding [7]. However, no consensus has been reached [8, 9]. In addition to t-PA, urokinase plasminogen activator (u-PA) has also been found to protect cerebral cortex neurons from soluble Aβ-induced synaptic damage [9, 10], but no association between the two has been found in experiments [4]. In addition, a small sample case-control study found that the plasminogen activator inhibitor-1 (PAI-1) level and PAI-1/t-PA ratio were significantly elevated in patients with mild cognitive impairment and AD [11–14]. It was also found in animal experiments that the expression of PAI-1 increased during aging or Aβ deposition, and clustered around amyloid plaques [4, 15]. An increase of intracellular or extracellular PAI-1 may promote the aging of brain cells in AD patients, and aging astrocytes could induce neuronal apoptosis by secreting pathologically active molecules [16]. However, animal experiments and observational studies do not allow causal inference, and thus, the relationship between plasminogen activators and their inhibitors and AD needs to be further confirmed.

Mendelian randomization (MR), which utilizes genotype as an instrumental variable (IV) to infer associations between phenotypes and disease, avoids reverse causality inference and reflects the long-term effects of exposure on outcomes. Therefore, this study intended to explore the relationship between plasminogen activators and their inhibitors and Alzheimer's disease through the MR method, so as to provide a reference for the prevention and control of Alzheimer's disease.

Material and methods

Three assumptions of MR

In MR research, genetic variation (single nucleotide polymorphism (SNP) is its most abundant form), which is strongly correlated with exposure factors, is selected as the instrumental variable, which refers to the exposure factors to be studied. By analyzing the association between genetic variation and exposure factors, and the association between genetic variation and outcome, the causal relationship between exposure factors and outcome could be inferred. The research needs to satisfy three assumptions: (1) the genetic variant is strongly associated with the exposure; (2) the genetic variant is independent of confounding factors of the association between exposure and outcome; (3) the genetic variant only affects the outcome via exposure. The graphical abstract shows the study procedure.

Data source

The tissue-type plasminogen activator phenotypes were extracted from 13 European ancestry cohorts up to 21,758 participants. The data provided by each cohort were fed into the 1000 Genomes Project phase 3 reference or later or to the Haplotype Reference Consortium (HRC) reference, which resulted in the testing of 21.4 M SNPs. A genome-wide association study (GWAS) was performed on two proteases – urokinase-type plasminogen activator and plasminogen activator inhibitor 1 – based on the INTERVAL study of European ancestry by Folkersen *et al.* [17]. The INTERVAL study included about 50,000 participants nested within a randomized trial of varying blood donation intervals. 2,731 and 831 participants were randomly selected from two non-overlapping sub-cohorts. A total of 3,301 participants (2,481 and 820 in the two sub-cohorts) remained for analysis after genetic quality control.

The summarized GWAS datasets for unspecified AD (Supplementary Table SI) were obtained from the FinnGen consortium (with genotype information for more than 16 million SNPs), which were curated, quality controlled, and harmonized in the IEU GWAS database (https://gwas.mrcieu.ac.uk/). The unspecified AD included 215,052 individuals, while the trait of unspecified AD (more controls excluded) excluded AD cases in its controls.

Data in the current study are publicly available and de-identified. Each GWAS involved obtained informed consent from participants, and received ethical approval from its respective institutions. Therefore, no ethical approval from the institutional review board (IRB) of the Sixth Hospital of Shanxi Medical University (General Hospital of Tisco) was required.

SNP screening methods

SNPs that are strongly correlated with exposure ($p < 5 \times 10^{-6}$) were selected to ensure that the MR correlation assumption (assumption 1) was satisfied; SNPs with LD were removed for being palindromic with intermediate allele frequencies (clump: $r^2 = 0.01$, kb = 1000).

Pleiotropy refers to a genetic variant being associated with multiple risk factors in different causal pathways. If a genetic variant used as an IV is additionally related to another risk factor for the outcome, then either the second or the third IV as-

sumption is violated, and the variant is not a valid IV. If pleiotropy leads to the genetic variant being associated with the outcome via a confounding variable, then the second assumption is violated. If pleiotropy leads to an alternative causal pathway from the variant to the outcome not via the exposure of interest, then the third assumption is violated.

The effect of SNPs on outcomes and exposure was harmonized so that both had the same allele. Outcome-related SNPs were removed from the IV (assumption 3).

To examine level pleiotropy, the removal of the IV may affect confounding SNPs through other exposures. Horizontal pleiotropy was tested using MR-Egger regression (assumption 2). "TwoSampleMR" (mr_pleiotropy_testfrom https://mrcieu.github.io/TwoSampleMR/articles/introduction.html#background R package).

Test

The strength of the association between the IV and exposure (assumption 1) was tested using $r^2 = 2 \times EAF \times (1 - EAF) \times b^2/SD^2$, $F = r^{2*}(N - 2)/(1 - r^2)$, MAF – minor allele frequency, b – beta, SD – standard difference; K – number of IVs; N – sample size.

Cochran's Q statistic has been used to study heterogeneity. This is a weighted sum of the squared distances of the variant-specific estimates from the overall IVW estimate.

$$Q = \sum_j se(\hat{\theta}_j)^{-2}(\hat{\theta}_{IVW} - \hat{\theta}_j)^2$$

Null hypothesis: each variant identifies the same causal parameter. J – Number of genetic variants

If heterogeneity exists, the results and conclusions obtained need to be treated with caution.

Statistical analysis

MR analysis: The main analysis method of MR is inverse-variance weighting (IVW), and the results are based on IVW analysis results. The weighted-median method and MR-Egger are the supplementary verifications for IVW results, and if they are consistent with the IVW results, the MR analysis results are considered to be statistically significant. In this study, two-way MR analysis was performed for statistically significant results of the main analysis. In addition, MR-PRESSO was used to test the pleiotropy of heterogeneity at the result level.

IVW: Based on the fixed-effects model, the Wald ratio method was used to obtain unconfounded estimates of the genetically predicted exposure's effect on outcome, which was the primary analysis for generating causal effect estimates in our study.

Weighted-median: A robust and consistent estimate of the effect was provided, even though nearly 50% of genetic variants were invalid instruments.

MR-Egger: A weighted linear regression was applied. However, the estimates of MR-Egger generally exhibited low precision and might be affected by outlying genetic variants.

MR-PRESSO (outlier tests): The MR-PRESSO analysis detected and attempted to reduce horizontal pleiotropy by removing significant outliers. But the MR-PRESSO outlier test required at least 50% of the genetic variants to be valid instruments, and relied on instrument strength independent of direct effect (InSIDE) assumptions.

MR-RAPS: A robust adjusted profile score was used. The profile likelihood of the Wald ratio (or ratio estimates) was maximized, accounting for weak instrument bias, pleiotropy and extreme outliers.

Results

SNP screening

First, the exposed SNPs were screened using $p < 5 \times 10^{-6}$, and SNPs meeting the conditions were screened out and counted (the second column of Table I). Then, the linkage imbalance was removed (the third column of Table I). Finally, SNPs related

Table I. Variable screening process

Variable	SNPs ($p < 5 \times 10^{-6}$)	Remove the presence of gene linkage imbalance (LD)	Correct strand for non-palindromic SNPs
Alzheimer's disease			
Tissue-type plasminogen activator levels	110	17	15
Urokinase-type plasminogen activator	181	16	10
Plasminogen activator inhibitor 1	317	11	17
Alzheimer's disease (more controls excluded)			
Tissue-type plasminogen activator levels	110	17	15
Urokinase-type plasminogen activator	181	16	10
Plasminogen activator inhibitor 1	317	11	17

Table II. Horizontal pleiotropy and heterogeneity of instrumental variables

Outcomes	Strength		MR-Egger intercept test		MR-PRESSO global test		Heterogeneity test		
	F value	R^2 (%)	Intercept	*P*-value	RSSobs	*P*-value	Q Egger	*P*-value	Q IVW	*P*-value
Alzheimer's disease										
Tissue-type plasminogen activator levels	114.45	0.005	-0.0632	0.5058	55.4015	0.595	11.3165	0.4171	11.8032	0.4616
Urokinase-type plasminogen activator	78.22	0.024	–0.0585	0.7243	56.6260	0.606	7.9887	0.3336	8.1426	0.4197
Plasminogen activator inhibitor 1	94.69	0.029	0.1035	0.4078	48.9106	0.645	5.3242	0.8685	6.0708	0.8686
Alzheimer's disease (more controls excluded)										
Tissue-type plasminogen activator levels	114.45	0.005	–0.0652	0.4958	54.6859	0.590	10.1838	0.5139	10.6799	0.5565
Urokinase-type plasminogen activator	78.22	0.024	–0.0675	0.6676	54.7432	0.665	6.2899	0.5063	6.4908	0.5924
Plasminogen activator inhibitor 1	94.69	0.029	0.0918	0.47	49.6764	0.606	5.8504	0.8277	6.4144	0.8443

to the outcome were matched, and the final SNPs were screened (the fourth column of Table I).

Main analyses

Correlation strength, pleiotropy, and heterogeneity testing was performed. The final calculated F-values were all greater than 10 (strength in Table II). This satisfied Mendelian randomization assumption 1. When the MR-Egger intercept test and MR- PRESSO global test were used for testing horizontal pleiotropy, all *p*-values were > 0.05 (Columns 4-5 in Table II), and the result showed no horizontal pleiotropy, this satisfied assumptions 2 and 3. Heterogeneity was not detected by the two heterogeneity test methods, and all the *p*-values were > 0.05 (columns 6–9 of Table II).

After the test was completed, MR analysis was started. In Alzheimer's disease, the IVW effect of plasminogen activator inhibitor 1 OR (95% CI) was 1.601 (1.068–2.400), and the interval did not contain 1 and $p = 0.0226$, which showed that plasminogen activator inhibitor 1 was positively correlated with the risk of Alzheimer's disease.

Sensitivity analyses

IVW model, weighted median, MR-PRESSO and MR-RAPS all showed similar results (OR value > 1). In the outcome of Alzheimer's disease (more controls excluded), the IVW effect of plasminogen activator inhibitor 1 OR (95% CI) was 1.543 (1.010–2.356), and the interval did not contain 1 and $p = 0.0448$. In conclusion, plasminogen activator inhibitor 1 was positively correlated with the risk of Alzheimer's disease (more controls excluded). IVW model, weighted median, MR-PRESSO and MR-RAPS all showed similar results (OR value > 1). Both outcomes were consistent (Table III).

A visual scatter plot was used to further illustrate the robustness of the positive results. The fit line (blue line) of the IVW in the graphical abstract was upward (i.e. the slope was positive). It further indicated that plasminogen activator inhibitor 1 was positively correlated with Alzheimer's disease and risk of Alzheimer's disease (more controls excluded). The more evenly scattered points were distributed on the left and right sides of the blue line, the less heterogeneity there was. Figure 1 further illustrates that there was no heterogeneity in the selected SNPs. Leave-one-out method results for sensitivity analysis of positive results are shown in Figure 2. "Leave one out" means to gradually remove each SNP, calculate the effect of the remaining SNPs, and observe whether the result changes after the removal of each SNP. If the result changes greatly after the removal of a SNP, it indicates that there is a SNP that has a great impact on the result, which we do not want to see. The aim is to prevent false positives in the posi-

Table III. MR results

Outcomes	Method	OR (95% CI)	*P*-value
Alzheimer's disease			
Tissue-type plasminogen activator levels	MR-Egger	3.451 (0.291–40.971)	0.3475
	Weighted median	1.022 (0.243–4.293)	0.9764
	Inverse-variance weighted	1.583 (0.545–4.601)	0.3987
	MR-RAPS	1.270 (0.429–3.759)	0.6655
	MR-PRESSO	1.219 (0.904–1.644)	0.2000
Urokinase-type plasminogen activator	MR-Egger	1.722 (0.384–7.723)	0.5009
	Weighted median	1.223 (0.601–2.490)	0.5781
	Inverse-variance weighted	1.326 (0.784–2.244)	0.2928
	MR-RAPS	1.445 (0.859–2.429)	0.1653
	MR-PRESSO	1.163 (0.901–1.502)	0.2498
Plasminogen activator inhibitor 1	MR-Egger	0.931 (0.243–3.573)	0.9196
	Weighted median	1.435 (0.704–2.922)	0.3201
	Inverse-variance weighted	1.601 (1.068–2.400)	0.0226
	MR-RAPS	1.092 (0.678–1.758)	0.7168
	MR-PRESSO	1.302 (1.004–1.688)	0.0519
Alzheimer's disease (more controls excluded)			
Tissue-type plasminogen activator levels	MR Egger	3.098 (0.254–37.846)	0.3949
	Weighted median	1.090 (0.246–4.837)	0.9097
	Inverse-variance weighted	1.380 (0.490–3.889)	0.5420
	MR-RAPS	1.081 (0.356–3.285)	0.8910
	MR-PRESSO	1.211 (0.896–1.636)	0.2186
Urokinase-type plasminogen activator	MR-Egger	1.875 (0.455–7.732)	0.4131
	Weighted median	1.375 (0.693–2.728)	0.3626
	Inverse-variance weighted	1.388 (0.865–2.227)	0.1739
	MR-RAPS	1.507 (0.891–2.550)	0.1265
	MR-PRESSO	1.165 (0.905–1.499)	0.2406
Plasminogen activator inhibitor 1	MR-Egger	0.954 (0.242–3.764)	0.9472
	Weighted median	1.476 (0.702–3.104)	0.3050
	Inverse-variance weighted	1.543 (1.010–2.356)	0.0448
	MR-RAPS	1.033 (0.636–1.677)	0.8955
	MR-PRESSO	1.268 (0.974–1.650)	0.0838

MR – Mendelian randomization, OR – odds ratio, CI – confidence interval.

tive results obtained because the effect of one or several SNPs is too strong. As shown in Figure 3, when any SNP was removed, the IVW result point estimates (black squares in the line segment) of the remaining SNPs were to the right of OR = 1 (the vertical gray line was the dividing line of OR = 1), that is, the OR value > 1. This further demonstrated the robustness of the results. The forest plots illustrated the causal relationship of PAI-1, t-PA and u-PA with AD, as shown in Figure 4.

Discussion

The present study was an MR study to explore the causal relationship between plasminogen activator, plasminogen activator inhibitor and AD. In this study, we found that genetically predicted PAI-1 expression was associated with an increased risk of AD, suggesting that PAI-1 might be one of the risk factors for AD at the genetic level.

PAI-1, t-PA, and u-PA are important active substances in the fibrinolytic system, and their dynamic balance plays an important role in the normal physiological function of the body's micro-vessels. AD, a progressive and degenerative brain disease, causes the occurrence of dementia [1, 18, 19]. AD pathology is characterized by the accumulation of amyloid, which consists of 39–43 amino acids cleaved from amyloid precursor protein (APP) [20, 21]. Aβ is neurotoxic and synaptically toxic, and the brain normally degrades and

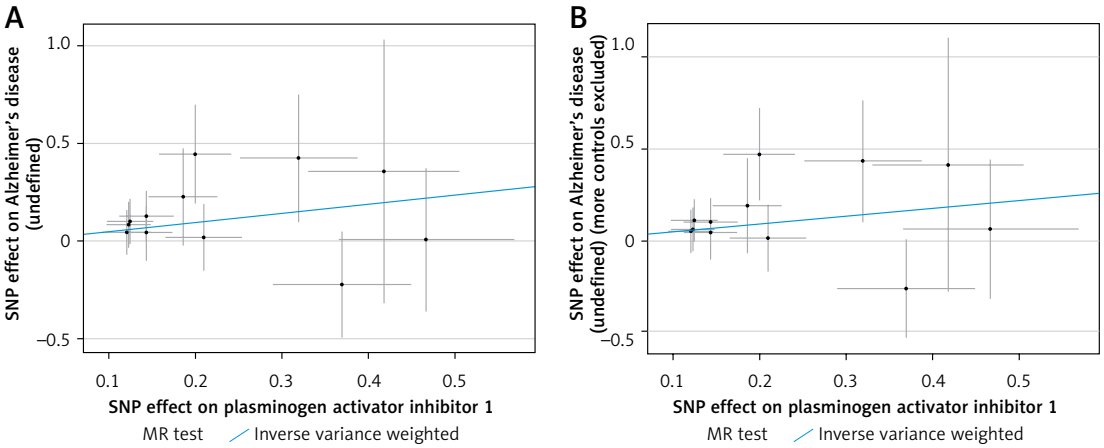

Figure 1. The scatter plot was drawn for positive results of plasminogen activator inhibitor 1 and the two outcomes. It can be seen that the blue line trends upward, indicating a positive correlation, which is consistent with the results shown in Table III

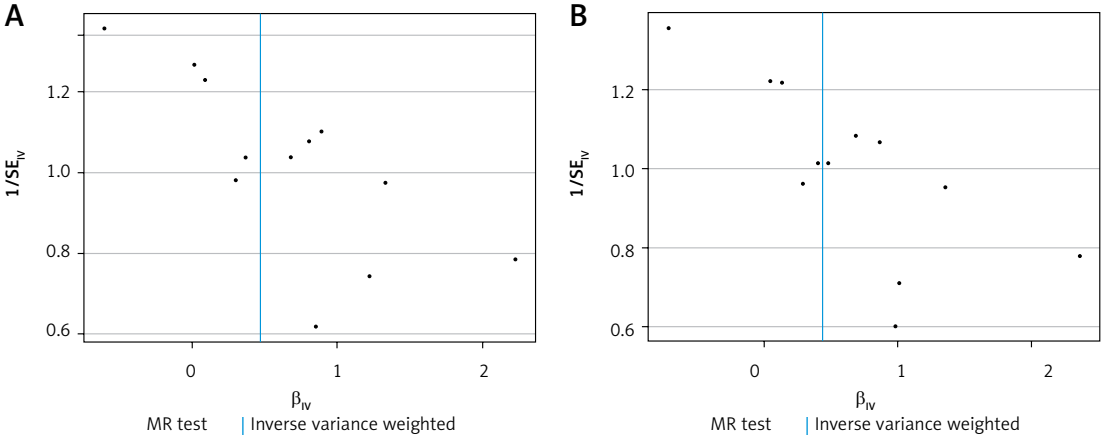

Figure 2. In the funnel plot, it can be seen that the blue line is in the middle of all SNP scatter points, indicating that the selection heterogeneity of SNPs is small

removes it [22]. An imbalance between Aβ production and clearance in the brain is central to the progression of AD. However, overproduction and deposition of Aβ result in early-onset familial AD, and decreased clearance of Aβ may be responsible for sporadic AD, which is more common than familial AD [23, 24]. Several Aβ degrading proteases have been identified as contributors to Aβ clearance, including insulin degrading enzymes, angiotensin converting enzymes, endothelin converting enzymes, neprilysins, and matrix metalloproteinases [25]. Tissue plasminogen activator (t-PA) can be involved in antibody clearance because it converts plasminogen to active plasmin, which is able to dissolve peptide fibrils [26, 27].

Earlier findings suggested that t-PA-activated plasmin may inhibit antibody aggregation and mitigate neurotoxicity. Under normal physiological conditions, PAI-1, an important inhibitor of the fibrinolytic pathway, inhibits u-PA and t-PA by forming complexes, thereby preventing the formation of plasmin [12, 28]. PAI-1 plays a variety of biological roles, including involvement in cell proliferation, apoptosis, adhesion, migration and signal transduction pathways. The expression of PAI-1 is regulated by many intrinsic (cytokines and growth factors) and extrinsic (cellular stress) factors. The gene encoding PAI-1 has multiple polymorphic sites, and the most studied site is 4G/5G polymorphism, which contains 4 or 5 (4G/5G) guanine bases at -675 of the PAI-1 promoters. When the PAI-1 gene is mutated, the level of PAI-1 increases, resulting in decreased fibrinolytic activity [11, 29, 30]. PAI-1 also plays a key role in various acute and chronic pathophysiological processes. It has been suggested that the imbalance of t-PA and PAI-1 can lead to thrombosis [15]. The level of PAI-1 in the blood of patients with cerebral infarction was significantly higher than that of normal people. In addition, the increase of blood PAI-1 level can be used as a predictor of recurrence of cerebral infarction, it is positively correlated with the recurrence of cerebral infarction, and the blood PAI-1 activity in patients with recurrent cerebral infarction is significantly higher than that in patients with primary cerebral infarction [31]. Cere-

bral infarction in animals is often accompanied by neurological symptoms, including convulsions, coma, and dyskinesia. If the site of cerebral infarction ruptures and causes intracranial hemorrhage, it can lead to death. Inhibition of PAI-1 expression has a thrombolytic effect, and it has certain guiding significance for predicting and treating cerebral infarction in animals [11, 23].

In this study, the genetic variation related to t-PA, u-PA, PAI-1 and AD was used as instrumental variables, and a Mendelian randomization study was conducted by screening the instrumental variables on the basis of the three basic assumptions, which helped avoid the influence of confounding factors and reverse causation. The causal association between t-PA, u-PA, PAI-1 and AD was better evaluated [32]. Secondly, the GWAS data sets on t-PA, u-PA, PAI-1 and AD are all from the largest data studies at present, and the very large sample size is also the advantage of this study. Finally, various methods were used to control the quality of the results, including the examination of the horizontal pleiotropy of the MR-Egger intercept, the treatment of weak instrumental variables by MR-RAPS and the analysis of sensitivity by the "leave-one-out" method, all of which ensured the robustness of the results and provided evidential support for the risk identification and prevention of Alzheimer's disease [33, 34].

In addition, it is worth noting that there are some limitations of this study. The biggest concern is the pleiotropy of genetic variation in the innate environment. Pleiotropy can be divided into vertical pleiotropy, which means that SNPs affect one trait (exposure) and then anoth-

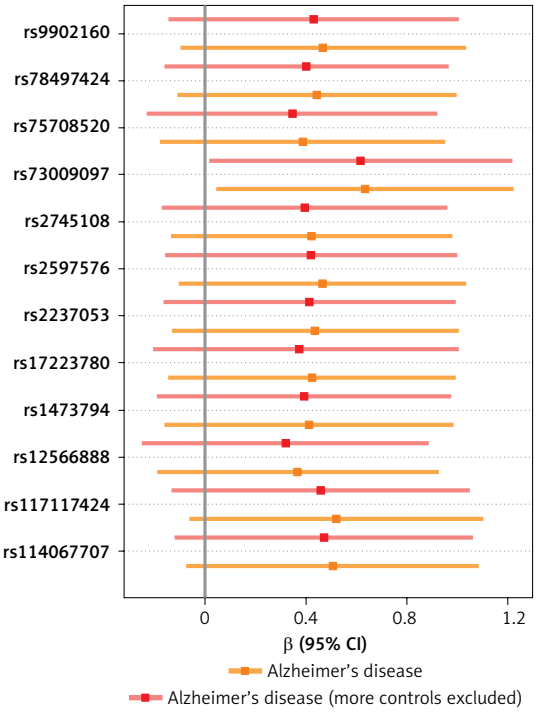

Figure 3 The leave-one-out method for sensitivity analysis showed that after removing each SNP one by one, each scatter center was greater than 0, which further demonstrated the positive correlation between plasminogen activator inhibitor 1 and Alzheimer's disease

Outcome and Exposure	Method	nSNPs	OR (95% CI)	*P*-value
Alzheimer's disease				
Plasminogen activator inhibitor 1	IVW (random effect)	17	1.601 (1.068–2.400)	0.023
	MR-Egger	17	0.931 (0.243–3.573)	0.920
	MR-RAPS	17	1.092 (0.678–1.758)	0.717
	Weighted median	17	1.435 (0.704–2.922)	0.320
	MR-PRESSO	17	1.302 (1.004–1.688)	0.052
Tissue-type plasminogen activator levels	IVW (random effect)	15	1.583 (0.545–4.601)	0.399
	MR-Egger	15	3.451 (0.291–40.971)	0.348
	MR-RAPS	15	1.270 (0.429–3.759)	0.666
	Weighted median	15	1.022 (0.243–4.293)	0.976
	MR-PRESSO	15	1.219 (0.904–1.644)	0.200
Urokinase-type plasminogen activator	IVW (random effect)	10	1.326 (0.784–2.244)	0.293
	MR-Egger	10	1.722 (0.384–7.723)	0.501
	MR-RAPS	10	1.445 (0.859–2.429)	0.165
	Weighted median	10	1.223 (0.601–2.490)	0.578
	MR-PRESSO	10	1.163 (0.901–1.502)	0.250
Alzheimer's disease (more controls excluded)				
Plasminogen activator inhibitor 1	IVW (random effect)	17	1.543 (1.010–2.356)	0.045
	MR-Egger	17	0.954 (0.242–3.764)	0.947
	MR-RAPS	17	1.033 (0.636–1.677)	0.896
	Weighted median	17	1.476 (0.702–3.104)	0.305
	MR-PRESSO	17	1.268 (0.974–1.650)	0.084
Tissue-type plasminogen activator levels	IVW (random effect)	15	1.380 (0.490–3.889)	0.542
	MR-Egger	15	3.098 (0.254–37.846)	0.395
	MR-RAPS	15	1.081 (0.356–3.285)	0.891
	Weighted median	15	1.090 (0.246–4.837)	0.910
	MR-PRESSO	15	1.211 (0.896–1.636)	0.219
Urokinase-type plasminogen activator	IVW (random effect)	10	1.388 (0.865–2.227)	0.174
	MR-Egger	10	1.875 (0.455–7.732)	0.413
	MR-RAPS	10	1.507 (0.891–2.550)	0.127
	Weighted median	10	1.375 (0.693–2.728)	0.363
	MR-PRESSO	10	1.165 (0.905–1.499)	0.241

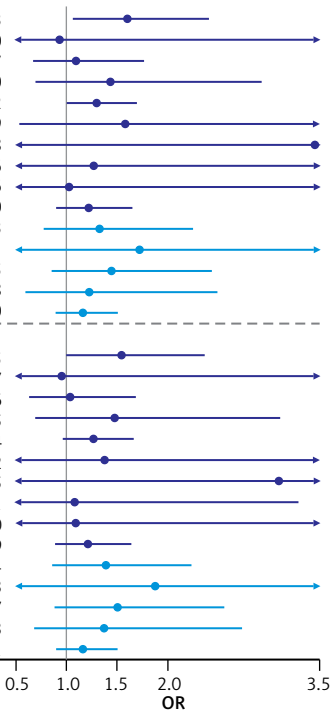

Figure 4. Association of PAI-1, t-PA and u-PA with AD

er (outcome), and horizontal pleiotropy, which means that SNPs affect both traits independently. Vertical pleiotropy can be tested by MR analysis, which should avoid horizontal pleiotropy. Because SNPs may affect both traits through independent pathways, it is difficult to demonstrate that vertical pleiotropy due to exposure is not biased. In other words, SNPs as instrumental variables may also influence outcomes in ways we do not yet know. Therefore, the multi-effect problem is tested by the MR-Egger intercept in this study, and the bias caused by it is reduced as much as possible. Secondly, the GWAS data used in this study came from Europe, which may limit the generality of our study to populations in other regions. Whether there is a linear association between PAI-1 and Alzheimer's disease needs to be further explored, and a possible threshold effect cannot be ruled out.

Conclusions

We explored the potential causal association of plasminogen activators and their inhibitors with AD. Our findings showed that genetically predicted PAI-1 expression was associated with an increased risk of AD. PAI-1 is a valuable marker for the occurrence of AD, which might provide new evidence for clinical intervention of AD.

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Ethical approval

Not applicable.

Conflict of interest

The authors declare no conflict of interest.

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