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Inflammatory factors mediated the effect of air pollution on ischemic stroke: a two-step, mediation Mendelian randomization study

Z.-L. HUANG[1], Z.-H. HUANG[1], Y. XIE[2], Y.-D. LI[1], Z.-D. PI[1], C. JIANG[1], A.-M. CHEN[3], X.-Y. GAO[3], J. WEN[1], J.-M. ZHU[1]

[1]Department of Neurology, Changde Hospital, Xiangya School of Medicine, Central South University, Changde, China
[2]Department of Neurology, Heyuan People's Hospital, Heyuan, Guangdong, China
[3]Department of Neurology, Zhujiang Hospital of Southern Medical University, Guangzhou, Guangdong, China

Zhilin Huang, Zhihua Huang and Ying Xie are co-first authors and contributed equally to this work

Abstract. – OBJECTIVE: Numerous investigations have indicated a correlation between air pollution (AP) and an elevated ischemic stroke (IS) likelihood. The existing literature does not provide a consensus about the possible link between AP and IS. A two-sample Mendelian randomization (MR) analysis was utilized to systematically measure the causal link between AP and ischemic stroke. Furthermore, the mediating impact of inflammatory factors was also performed by a two-step MR.

MATERIALS AND METHODS: A two-sample MR analysis was utilized to examine the AP impact on the incidence of IS. Additionally, a two-step MR approach was carried out to account for possible mediating variables. The indirect impact was determined by employing the product approach, which included multiplying the AP impact on inflammatory factors by the inflammatory factors' impacts on IS. The MR effect was identified through inverse variance-weighted (IVW) meta-analysis of each Wald Ratio. Additionally, complementary studies were conducted using the weighted median and MR-egger approaches.

RESULTS: The IVW method with random effects showed that the per unit increase in genetically predicted PM2.5 was linked to the 0.362-fold elevated ischemic stroke risk (OR: 1.362, 95% CI: 1.032-1.796, *p*=0.029). Furthermore, the IVM technique, incorporating random effects, demonstrated that the per unit increase in genetically predicted PM2.5 was related to an elevated Interleukin (IL)-1β risk (OR: 1.529, 95% CI: 1.191-1.963, *p*=0.001), IL-6 (OR: 1.498, 95% CI: 1.094-2.052, *p*=0.012) and IL-17 (OR: 1.478, 95% CI: 1.021-2.139, *p*=0.038). IL-1β, IL-6, and IL-17 modulated the PM2.5 impact on ischemic stroke, while the proportion mediated by them was 59.5%.

CONCLUSIONS: A positive correlation between genetically predicted PM2.5 levels and elevated ischemic stroke risk is mediated by IL-1β, IL-6, and IL-17.

Key Words:
Air pollution, Inflammatory factors, Ischemic stroke, Mendelian randomization, Mediation effect.

Introduction

Stroke is a significant issue in global health, as it holds the position of the second most prevalent cause of mortality and the third most common cause of adult disability. Consequently, stroke poses substantial burdens on both society and the economy[1]. According to the GBD 2019 study[2], the prevalence of stroke in China escalated to 276.7 per 100,000 individuals in 2019, exhibiting a notable rise of 86.0% compared to the rate recorded in 1990. Ischemic stroke (IS) comprises about 80% of stroke occurrences and is characterized as a complicated and heterogeneous neurological condition with diverse etiologies[3]. Previous studies[4] showed that hypertension, advanced age, obesity, diabetes, atrial fibrillation, and dyslipidemia are recognized as the established likelihood factors for stroke.

Air pollution (AP) poses a persistent global public health issue for the general population[5].

Corresponding Authors: Jianming Zhu, MD; e-mail: zhujm0718@126.com;
Jun Wen, MD; e-mail: cdwenjun1973@163.com;
Xiaoya Gao, MD; e-mail: gaoxy23@gmail.com

The presence of AP has been correlated to many respiratory ailments, encompassing chronic obstructive pulmonary disease, acute lower respiratory infection, and lung cancer[6], in addition to cardiovascular diseases[7,8]. Previous studies[9,10] have shown that the increased risk of stroke varied from 0.26 to 0.80% with 10 μg/m^3 elevation in PM2.5. Nevertheless, there remains a lack of agreement regarding the link between AP and IS risk. As an illustration, empirical investigations[11] have indicated that the link between AP and stroke mortality ceased to exist after 2007, coinciding with a sustained decrease in AP levels. In another case-control study[12], it was observed that there was an increase in the occurrence of stroke in patients. Furthermore, the precise causal link between AP and IS and the underlying mechanism driving this association remains uncertain.

Notably, clinical investigations[13,14] have demonstrated that AP has the potential to influence IS functioning through the modulation of the levels of inflammatory factors, including IL-1β and Interleukin-6 (IL-6). Hence, it was postulated that potential causal links could exist between AP, inflammatory markers, and the occurrence of IS. Hence, we elucidated these links and ascertained possible metabolites that may serve as early diagnostic markers and targets for clinical intervention. Mendelian randomization (MR) is a commonly employed method in research, which utilizes genetic variants as instrumental variables (IVs) to effectively control the influence of any confounding factors[15], which can mitigate the issue of reverse causation bias, hence enabling stronger causal inferences to be made regarding the link between exposure and clinical outcomes. The maintenance of genetic variation is of utmost importance, necessitating mitigating confounding factors and reducing reverse causation risks[16]. Therefore, MR comprehensively understands the causal link between exposure and result. Furthermore, employing a two-step MR can be a highly successful approach for investigating the presence of mediating effects[17]. In the current research, we carried out a two-step MR research and two mediation analyses employing summary statistics acquired from the most extensive and latest genome-wide association studies (GWAS) that were accessible for the study. Our aim was to investigate the links between IS, AP, and inflammatory variables by dissecting their correlations.

Materials and Methods

Study Design and Data Retrieval

In this research investigation, we conducted a two-sample MR analysis to evaluate the effect of various air pollutants [PM2.5, PM10, Nitrogen dioxide (NO_2), Nitrogen oxides (NOx)] on IS. To ensure the mediating factors, we subsequently performed a two-step MR. Firstly, we conducted an assessment of the link between AP and inflammatory variables. Second, we explored the causal effect of inflammatory variables on IS. The schematic representation of the present investigation design is depicted in Figure 1.

Summary single nucleotide polymorphism (SNP)–phenotype-related information was acquired from different GWAS[18]. Publicly available summary data for PM2.5, PM10, NO_2, and NOx were obtained from the UK Biobank, comprising 304,818 participants of European ancestry[19]. The UK Biobank is an all-encompassing biological database and research resource that incorporates a wide range of genetic and health data obtained from a cohort of 500,000 people in the UK (https://www.ukbiobank.ac.uk/). Indicators related to AP were measured using land use regression (LUR) models[20].

The investigation obtained the summary statistics for IL-1β, IL-18, IL-6, and IL-17 from a GWAS conducted by the Systematic and Combined AnaLysis of Olink Proteins (SCALLOP) collaboration[21]. The GWAS included a total of 21,758 individuals of European ancestry[21]. The summary statistics pertaining to IL-16 were obtained from the GWAS conducted on The Cardiovascular Risk in Young Finns Study (YFS) and FINRISK datasets[22].

Furthermore, data for IS was obtained from the MEGASTROKE consortium[23] of 520,000 subjects, which found 32 loci linked to stroke and stroke subtypes. The MEGASTROKE working group has undertaken the task of consolidating all existing GWAS on stroke, both within the International Stroke Genetics Consortium (ISGC) and outside. Their objective was to conduct a meta-analysis of genome-wide association across many ancestries (https://www.strokegenetics.org/node/317). All the information is presented in Table I.

Genetic Instrumental Variables Selection and Data Harmonization

As instruments, the MR study employed genetic variants that achieved genome-wide significance

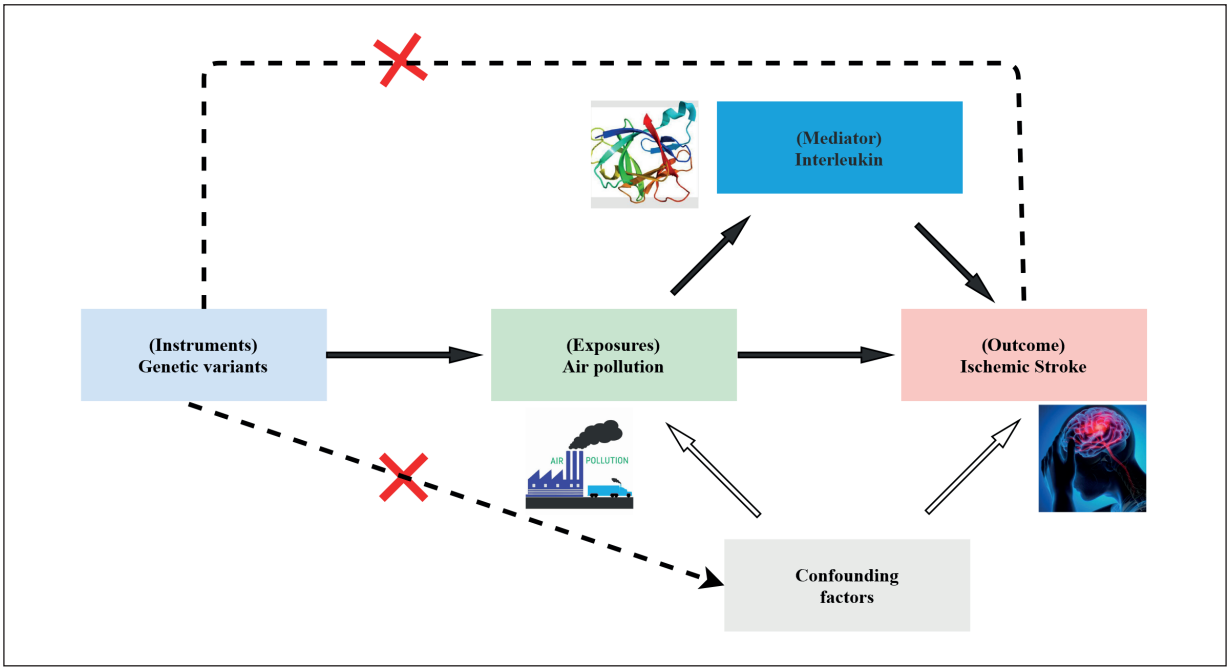

Figure 1. The overview of the present MR for the association between air pollution and ischemic stroke.

($p<5 \times 10^{-8}$) for PM10, NO_2, and NOx. Furthermore, we have reduced the genome-wide significance threshold for PM2.5 and inflammatory components to $p<5\times 10^{-6}$ to guarantee that a sufficient number of SNPs is selected as IVs[24-26]. The linkage disequilibrium was assessed to ascertain the presence of any SNPs that exhibited a state of linkage disequilibrium. To achieve this, SNPs within a 10,000 kb window were pruned depending on a threshold of $R^2<0.001$, ensuring that the SNPs were independent[27]. And the R^2 and Kb for PM2.5 and inflammatory factors were 5,000 and 0.01, respectively. After that, SNPs associated with any confounding factor and IS were also excluded. Additionally, we performed SNP harmonization in order to rectify the orientation of alleles. The final IVs of AP and inflammatory factors are presented in the **Supplementary Table I**.

MR Analysis and Mediation Analysis

The total impact of a given exposure on a certain result can be analyzed by breaking it down into two distinct components: indirect effects and direct impacts[28]. The total impact of the AP on IS was abstained by two-sample MR. The meta-analysis of each Wald Ratio was carried out employing the inverse variance-weighted (IVW) technique, which is often regarded as the most

Table I. Details of the Genome-Wide Association Studies included in this Mendelian randomization analysis.

Exposures/Outcomes		Consortium	Ethnicity	Participants	Sex
Air pollution	PM2.5 um	UK Biobank	European	423,796	Males and females
	PM10 um	UK Biobank	European	455,314	Males and females
	Nitrogen dioxide	UK Biobank	European	456,380	Males and females
	Nitrogen oxides	UK Biobank	European	456,380	Males and females
Mediating factors	Interleukin 1β	SCALLOP consortium	European	21,758	Males and females
	Interleukin 6	SCALLOP consortium	European	21,758	Males and females
	Interleukin 16	YFS/FINRISK	European	3,483	Males and females
	Interleukin 17	SCALLOP consortium	European	21,758	Males and females
	Interleukin 18	SCALLOP consortium	European	21,758	Males and females
Outcomes	Ischemic stroke	MEGASTROKE consortium	European	440,328	Males and females

YFS, Cardiovascular Risk in Young Finns Study; PM, Particulate matter.

reliable methodology in situations when there is a lack of evidence indicating the existence of directional pleiotropy[29]. The IVW with random impact or fixed effect was based on the heterogeneity test. The random-impacts IVM model was used in cases when significant heterogeneity was identified. Conversely, the fixed-effect IVW model was carried out[30]. We conducted complementary analyses using the weighted median and MR-egger method[31]. In the MR-Egger method, the gene-exposure coefficients are utilized to regress the gene-outcome coefficients in a weighted linear regression analysis[32]. We performed the two-step MR to explore whether inflammatory factors mediated the AP impact on IS. The indirect impact was determined by multiplying the AP impact on inflammatory factors and the inflammatory factors' impact on IS[33] by employing the product method. The MR impact of inflammatory factors on IS adjusted for genetically determined AP was obtained by including the genetic proxies in the multivariable MR analysis[33,34]. The estimation of the mediation impact proportion was conducted employing the following equation:

Sensitivity Analysis

Furthermore, we conducted the MR pleiotropy residual sum and outlier test (MR-PRESSO) to find and address any potential impacts caused by outliers[35]. The MR-Egger approach was utilized to measure the possible impacts of pleiotropic SNPs. If the *p*-value of the intercept was >0.05, it indicated the existence of significant pleiotropy[36]. The heterogeneity among SNPs was assessed employing Cochrane's Q value. The scatter plots and funnel plots were also performed to do the sensitivity analysis. If the funnel plots were symmetrical, the results were stable.

Statistical Analysis

The analyses were conducted employing the packages "Two-Sample-MR" (version 0.5.6) and "MR-PRESSO" (version 1.0) in the statistical R (version 4.0.5, Auckland, New Zealand). Statistical significance was determined by *p*-values<0.05.

Results

The Total Effect of AP on IS

Table II shows the link between AP and IS. After IV selection, 49, 19, 5, and 8 SNPs remained for PM2.5, PM10, NO_2, and NOx. The IVW method with random effects showed that the per unit increase in genetically predicted PM2.5 was linked to the 0.362-fold elevated IS risk (OR: 1.362, 95% CI: 1.032-1.796, p=0.029). The link was also found in the sensitivity analyses employing IVW with fixed effect (OR: 1.362, 95% CI: 1.077-1.722, p=0.010). There was heterogeneity but no horizontal pleiotropy in this MR analysis ($p_{heterogeneity}$=0.039, $p_{horizontal\ pleiotropy}$=0.211, Table III). The scatter plot and funnel plot are presented in Figure 2.

However, the IVW method showed that the per unit increase in genetically predicted PM10, NO_2, and NOx was not linked to the elevated risk of IS (p_{PM10}=0.736, p_{NO_2}=0.067, p_{NOx}=0.162). Detailed information on heterogeneity and horizontal pleiotropy is presented in Table III.

The Effect of Inflammatory Factors on IS

The IVW method with fixed effect indicated that the per unit increase in genetically predicted IL-1β (OR: 1.052, 95% CI: 1.005-1.101, p=0.028), IL-6 (OR: 1.086, 95% CI: 1.009-1.168, p=0.027) and IL-17 (OR: 1.140, 95% CI: 1.021-1.273, p=0.020) was linked to the elevated risk of IS. The findings remained consistent across the sensitivity analysis using IVW with random effect (Table IV). The MR analysis did not exhibit heterogeneity or horizontal pleiotropy (Table III). However, null effects were found on IL-16 and IL-18 on IS (p_{IL-16}=0.938, p_{IL-18}=0.601).

Mediation Effect and Proportion by IL-1β, IL-6 and IL-17

Table II also reported the association between inflammatory factors and IS.

The IVW method with random effect showed that the per unit increase in genetically predicted PM2.5 was linked to the 0.529-fold elevated IL-1β risk (OR: 1.529, 95% CI: 1.191-1.963, p=0.001). The link was also found in the sensitivity analyses using IVW with fixed effect (OR: 1.529, 95% CI: 1.191-1.963, p=0.001) and weighted median method (OR: 1.648, 95% CI: 1.144-2.373, p=0.007). This MR investigation did not exhibit any heterogeneity or horizontal pleiotropy (Table III).

Furthermore, the IVW method with random impact revealed that the per unit increase in genetically predicted PM2.5 was linked to the elevated IL-6 (OR:1.498, 95% CI: 1.094-2.052, p=0.012) and IL-17 (OR: 1.478, 95% CI: 1.021-

Table II. The association between air pollution and ischemic stroke and inflammatory factors by two-sample MR analysis.

Exposure	Outcome	MR				
		nSNP	Methods	Beta	OR (95% CI)	*p*-value
PM2.5	Ischemic stroke	49	IVW (re)	0.309	1.362 (1.032,1.796)	0.029
			IVW (fe)	0.309	1.362 (1.077,1.722)	0.010
			MR-Egger	-0.196	0.822 (0.360,1.880)	0.645
			Weighted median	0.162	1.176 (0.829,1.668)	0.364
PM10	Ischemic stroke	19	IVW (re)	0.076	1.079 (0.694,1.676)	0.736
			IVW (fe)	0.076	1.079 (0.777,1.498)	0.651
			MR-Egger	-0.544	0.580 (0.154,2.180)	0.431
			Weighted median	-0.415	0.660 (0.391,1.114)	0.120
Nitrogen dioxide	Ischemic stroke	5	IVW (re)	0.584	1.794 (0.959,3.356)	0.067
			IVW (fe)	0.584	1.794 (0.959,3.356)	0.067
			MR-Egger	0.832	2.297 (0.141,4.427)	0.296
			Weighted median	0.493	1.637 (0.758,3.537)	0.210
Nitrogen oxides	Ischemic stroke	8	IVW (re)	-0.392	0.675 (0.389,1.173)	0.164
			IVW (fe)	-0.392	0.675 (0.390,1.171)	0.162
			MR-Egger	0.077	1.080 (0.061,19.127)	0.960
			Weighted median	-0.117	0.890 (0.434,1.826)	0.751
PM2.5	IL-1β	59	IVW (re)	0.425	1.529 (1.191,1.963)	0.001
			IVW (fe)	0.425	1.529 (1.191,1.963)	0.001
			MR-Egger	0.390	1.477 (0.871,2.505)	0.153
			Weighted median	0.499	1.648 (1.144,2.373)	0.007
PM2.5	IL-6	59	IVW (re)	0.404	1.498 (1.094,2.052)	0.012
			IVW (fe)	0.404	1.498 (1.094,2.052)	0.012
			MR-Egger	0.316	1.372 (0.574,3.277)	0.480
			Weighted median	0.436	1.547 (0.988,2.423)	0.057
PM2.5	IL-17	39	IVW (re)	0.391	1.478 (1.021,2.139)	0.038
			IVW (fe)	0.391	1.478 (1.021,2.139)	0.038
			MR-Egger	0.306	1.358 (0.758,2.433)	0.310
			Weighted median	0.312	1.366 (0.772,2.417)	0.284

PM, Particulate matter; IL, interleukin; SNP, single nucleotide polymorphism; OR, odds ratio; MR, Mediation Mendelian; re, random effect; fe, fixed effect.

Table III. Heterogeneity and pleiotropy test of this MR analysis.

Exposure	Outcome	Heterogeneity		Pleiotropy	
		Cochran's Q	*p*-value	Egger-intercept	*p*-value
PM2.5	Ischemic stroke	66.613	0.039	0.008	0.211
PM10	Ischemic stroke	32.418	0.020	0.012	0.344
Nitrogen dioxide	Ischemic stroke	1.372	0.849	-0.021	0.443
Nitrogen oxides	Ischemic stroke	7.056	0.423	-0.007	0.755
IL-1β	Ischemic stroke	19.913	0.339	-0.007	0.302
IL-6	Ischemic stroke	3.381	0.908	0.003	0.727
IL-16	Ischemic stroke	21.025	0.007	-0.006	0.618
IL-17	Ischemic stroke	4.786	0.310	-0.019	0.397
IL-18	Ischemic stroke	92.200	0.000	0.011	0.259
PM2.5	IL-1β	56.312	0.538	0.001	0.885
PM2.5	IL-6	43.084	0.928	0.001	0.832
PM2.5	IL-17	22.853	0.975	0.002	0.716

PM, Particulate matter; IL, interleukin.

2.139, p=0.038) risk. There was no heterogeneity and horizontal pleiotropy in this MR analysis (Table III).

A multivariable MR was carried out to evaluate the direct impact of the exposure variable on the outcome variable while controlling for

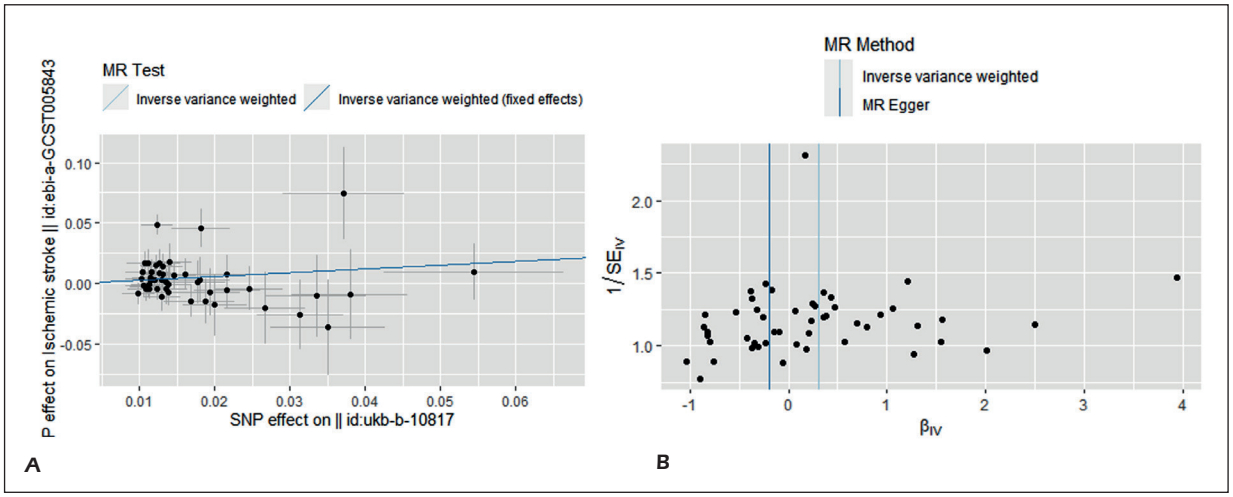

Figure 2. **A**, The scatter plot for the MR of air pollution and ischemic stroke. **B**, The funnel plot for the MR of air pollution and ischemic stroke.

potential mediating factors. Table V presents the mediation analysis of the PM2.5 impact on IS. In the multivariable MR analysis of PM2.5–IL-1β–ischemic stroke, the direct effect was shown to be diminished, with an OR of 1.202. The percentage of mediation attributed to IL-1β was 6.1%. In the multivariable MR analysis of the link between PM2.5, IL-6, and IS, the observed direct impact was found to be diminished, resulting in an OR of 1.151. The percentage of mediation attributed to IL-6 was 4.6%. The multivariable MR analysis of the link between PM2.5, IL-17, and IS revealed that the direct influence was weakened, resulting in an OR of 1.252. The

Table IV. The association between inflammatory factors and ischemic stroke.

Exposure	Outcome	MR				
		nSNP	Methods	Beta	OR (95% CI)	*p*-value
IL-1β	Ischemic stroke	19	IVW (re)	0.051	1.052 (1.003,1.104)	0.037
			IVW (fe)	0.051	1.052 (1.005,1.101)	0.028
			MR-Egger	0.099	1.104 (0.998,1.222)	0.071
			Weighted median	0.059	1.060 (0.994,1.132)	0.076
IL-6	Ischemic stroke	9	IVW (re)	0.082	1.086 (1.009,1.168)	0.027
			IVW (fe)	0.082	1.086 (1.009,1.168)	0.027
			MR-Egger	0.057	1.059 (0.907,1.236)	0.494
			Weighted median	0.089	1.094 (1.003,1.193)	0.043
IL-16	Ischemic stroke	9	IVW (re)	-0.002	0.998 (0.956,1.042)	0.938
			IVW (fe)	-0.002	0.998 (0.972,1.025)	0.900
			MR-Egger	0.014	1.014 (0.941,1.094)	0.721
			Weighted median	-0.013	0.987 (0.955,1.019)	0.419
IL-17	Ischemic stroke	5	IVW (re)	0.131	1.140 (1.021,1.273)	0.020
			IVW (fe)	0.131	1.140 (1.030,1.261)	0.011
			MR-Egger	0.261	1.298 (0.980,1.719)	0.167
			Weighted median	0.123	1.130 (0.996,1.283)	0.058
IL-18	Ischemic stroke	26	IVW (re)	0.021	1.022 (0.943,1.107)	0.601
			IVW (fe)	0.021	1.022 (0.980,1.065)	0.315
			MR-Egger	-0.060	0.942 (0.803,1.105)	0.467
			Weighted median	-0.034	0.967 (0.903,1.034)	0.327

IL, interleukin; SNP, single nucleotide polymorphism; OR, odds ratio; MR, Mediation Mendelian; re, random effect; fe, fixed effect.

Table V. Mediation analysis of the effect of PM2.5 on ischemic stroke by multivariable MR analysis.

Exposure/Outcome	Adjusted factors	Direct effect		Mediation effect		
		β	OR	β	OR	Mediation effect (%)
PM 2.5/Ischemic stroke	Interleukin 1β	0.183	1.202	0.019	1.019	6.1
PM 2.5/Ischemic stroke	Interleukin 6	0.141	1.151	0.014	1.014	4.6
PM 2.5/Ischemic stroke	Interleukin 17	0.225	1.252	0.125	1.133	40.4
PM 2.5/Ischemic stroke	Interleukin 1β, Interleukin 6 and Interleukin 17	0.036	1.037	0.184	1.202	59.5

PM, Particulate matter; IL, interleukin; OR, odds ratio.

percentage of the effect that was mediated by IL-17 was found to be 40.4%. The percentage of mediation attributed to IL-1β, IL-6, and IL-17 was measured to be 59.5%.

Discussion

We used the summary data from a comprehensive GWAS to examine the causal link between AP and IS. The findings of this study revealed a possible connection between genetically determined AP and increased levels of inflammatory factors and IS risk. The present MR study has indicated a positive link between elevated levels of AP and an IS risk. Meanwhile, this study has demonstrated that the elevated IL-1β, IL-6, and IL-17 risk may influence the heightened susceptibility to AP and IS.

AP has been found[37] to expedite the progression of atherosclerosis, hence heightening the thrombosis risk and subsequently raising the occurrence of acute coronary syndrome and stroke. Previous research[38,39] has shown compelling evidence supporting a robust link between AP and the stroke mortality rate. According to a retrospective study conducted by the GBD[40] in 2019, there has been a notable rise in the incidence of stroke cases linked to PM2.5 over a span of 30 years. Nevertheless, certain investigations have failed to construct a statistically significant correlation between AP and the occurrence of IS. The outcomes of an observational study[11] indicated an absence of a significant connection between the levels of AP and mortality rates pertaining to various subtypes of stroke. Although there is a connection between AP and IS, the findings from different epidemiological research have not yielded consistent results. The observed disparities in the outcomes may be ascribed to a range of factors, encompassing the magnitude and duration of AP and the adsorbed constituents of AP. These variables collectively exert an influence on the AP impacts on the IS[41].

Our study utilizing MR presents genetic evidence supporting the involvement of several inflammatory variables in mediating the causal effects of AP on the incidence of IS. Greater levels of IL-1β, IL-6, and IL-17 were linked to an elevated IS risk in the present investigation. A large case-control investigation[42] demonstrated that high levels of IL-1β and IL-6 were linked to a higher risk of stroke. Together, these inflammatory cytokines promoted more leukocytes, thereby enlarging the cerebral infarct area[43]. Consistent with this, another study[44] found that IL-17 is detrimental to stroke. In addition, IL-17 promotes inflammatory responses by activating the nuclear factor-kappaB (NF-κB) and mitogen-activated protein kinases (MAPK) signaling pathways[45,46]. These pathways are known to be activated in the context of IS and are considered the primary mechanisms underlying the inflammatory response. The aforementioned data underscores the potential of selectively modulating inflammatory factors as a viable approach to enhance the treatment of IS. Therefore, further investigation is needed to better understand the complex link between inflammatory factors and IS in future research endeavors.

The mediation analyses yielded genetic evidence supporting a connection between AP and inflammatory variables. Furthermore, our outcomes indicate that the heightened risk of AP in relation to IS may be influenced by a higher susceptibility to IL-1β, IL-6, and IL-17. An increasing body of evidence indicates that AP is

responsible for the generation of pro-inflammatory signals beginning in peripheral tissues and organs, resulting in a cytokine response induced by the systemic system[13,47-49], which subsequently triggers inflammation within the brain. Exposure to particulate matter has the potential to increase the concentration of plasma cytokines, such as IL-1β and IL-6. It is postulated that these cytokines are released within the circulatory system due to the interplay between particles, alveolar macrophages, and airway epithelial cells[14]. IL-1β serves as a prominent pro-inflammatory cytokine implicated in the inflammatory response, namely in its disruption of the blood-brain barrier[50]. The inhibition of IL-1β activity has the potential to mitigate ischemic brain injury[51]. Hence, these outcomes, in conjunction with the outcomes of our investigation, offer valuable perspectives on the causal link between AP and inflammatory markers.

Our research possesses several strengths in the domain of causal inference. Firstly, the utilization of MR design offers several advantages compared to typical observational research. This approach effectively mitigates the risk of potential unmeasured confounders and reverse causation. Initially, an MR study was conducted with the purpose of evaluating the probable causal link between AP and IS. This approach effectively mitigates the inherent constraints commonly seen in conventional observational research, such as confounding factors related to the environment, reverse causality, inadequate sample sizes, and others. Secondly, using GWAS data greatly improves the statistical ability to detect causal effects by having sufficient cases. Thirdly, the genetic variation of IVs is distributed over distinct chromosomes, which serves to reduce the potential influence of gene-gene interactions on the calculated values. Fourthly, we conducted a mediation analysis employing a two-step MR approach. Our findings suggest that the relationship between AP and IS may be mediated by inflammatory factors.

However, the study also has some limitations. First, we identified that inflammatory variables significantly affect IS development. However, the correlation between AP and the occurrence of IS appears to possess a very intricate nature. Further research is urgently needed to explore additional significant pathways linking AP with IS, including particle effects and adsorbed compounds in AP[41]. Secondly, summary-level data restricted our ability to include non-linear MR studies and conduct subgroup analyses. Furthermore, the MR evaluated the causal effect of lifelong exposure to likelihood variables on various outcomes. Assessing the efficacy of an intervention targeting exposure within a designated timeframe poses inherent challenges[52]. Thirdly, the existing studies failed to account for the reciprocal relationship between AP and IS. Fourthly, we did not explore the association between air pollution and different stroke subtypes. In addition, it should be noted that the study predominantly consisted of participants of European ancestry, hence constraining the outcome's generalizability to other demographic cohorts. Finally, MR assumes a linear link between exposure and result. However, it is crucial to acknowledge that, in reality, this association may exhibit a more intricate nature, encompassing non-linear connections and interactions with additional environmental and genetic elements[53]. Hence, it is imperative to thoroughly examine any non-linear and interaction impacts between AP and IS in forthcoming MR research.

Conclusions

Genetically predicted PM2.5 was positively linked to elevated IS risk, with evidence that IL-1β, IL-6, and IL-17 mediated the impact of it. More work is needed for policy formulation to decrease AP and the emission of toxic and harmful gases.

Informed Consent

The authors declare that they have no conflict of interests.

Ethics Approval

Not applicable.

Conflict of Interest

There was no conflict of interest.

Authors' Contributions

All authors participated equally in this MR study.

Funding

None.

Availability of Data and Materials

The data analyzed in this MR are presented in the **Supplementary Table I**.

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