Causal Effects of PM$_{2.5}$, NO$_x$, and NO$_2$ on Cognitive Function: A Two-sample Mendelian Randomization Study

Yilin Tang, Zhenyu Li, Yicheng Fu, Mingyi Zhao*

Department of Pediatrics, The Third Xiangya Hospital, Central South University, Changsha, Hunan 410013, China

ABSTRACT

Epidemiological studies have revealed that air pollutants are related to cognitive decline, but a causal relationship has not been established. We conducted a two-sample Mendelian randomization (MR) study using pooled statistics from publicly available genome-wide association study (GWAS) data to show the association between air pollutants and cognitive decline in the European population. The exposure factors in our analysis were air pollutants, including "particulate matter air pollution (PM$_{2.5}$); 2010", "nitrogen oxides air pollution; 2010" and "nitrogen dioxide air pollution; 2010", while our outcome variables focused on cognitive function, including the "fluid intelligence score", "mean time to correctly identify matches", and "number of incorrect matches in the round". We applied various MR methods, including inverse-variance weighted (IVW), weighted median, and MR-Egger regression, to estimate the causal effects. Furthermore, we conducted heterogeneity and pleiotropy tests to ensure the robustness of our findings. Our study revealed a significant negative correlation between NO$_x$ and fluid intelligence scores ($\beta$ -0.879, 95% CI [–1.423, –0.336], $p$ = 0.002), indicating that among air pollutants, NO$_x$ has a detrimental impact on cognitive function. No significant associations were found between PM$_{2.5}$ or NO$_2$ and cognitive function. The presence of NO$_x$ is associated with a decrease in the fluid intelligence score, suggesting adverse effects of NO$_x$ on logic and reasoning skills. These findings further emphasize the importance of preventing and treating air pollution exposure and suggest that early cognitive screening in people exposed to air pollution can prevent the development of neurodegenerative diseases.

Keywords: PM$_{2.5}$, NO$_2$, NO$_x$, Cognitive function

1 INTRODUCTION

In recent years, the problem of air pollution has become increasingly serious and has gradually attracted the attention of public health departments (Sin et al., 2023). The detrimental effects of air pollution on human health have been realized. In the past, the focus of attention was on the respiratory and cardiovascular systems, but in recent years, it has been found that the nervous system is also inevitably affected (Landrigan et al., 2005; Weisenberg et al., 2020; Dong et al., 2023). The toxic effects of air pollutants on the nervous system include neurological damage and mental illness, and mental illness includes a series of diseases associated with cognitive dysfunction, such as Alzheimer's disease and Parkinson's disease (Ghorani-Azam et al., 2016). In the 2020 Lancet Commission report, air pollution was included as a new modifiable risk factor for dementia (Livingston et al., 2020). Air pollution is thought to be responsible for tau and β-amyloid deposition, stroke and cardiovascular disease, oxidative stress, neuroinflammation, and changes in dopamine and glutamate levels, leading to cognitive decline (Allen et al., 2017; Schikowski and Altug, 2020).

PM$_{2.5}$ (Particulate matter 2.5) is a globally recognized environmental pollutant that affects human health and has harmful effects on multiple systems, including the respiratory system, cardiovascular system, and nervous system (Wang et al., 2021b). In a cross-sectional study conducted in Los Angeles,
PM$_{2.5}$ was found to be negatively correlated with speech learning and brain function (Gatto et al., 2014). A cross-sectional study conducted in the UK also revealed a positive correlation between the PM$_{2.5}$ concentration and the severity of mental disorders (Hao et al., 2022). Epidemiological neuroimaging studies have also indicated that there seems to be a connection between increased PM$_{2.5}$ concentrations and increased Aβ cerebral plaques in the brains of elderly people with cognitive impairment (Iaccarino et al., 2021). Similarly, an increased Aβ load was found in individuals exposed to high PM$_{2.5}$ concentrations in a Chinese cohort (Ma et al., 2023b). In mouse experiments, researchers have shown that PM$_{2.5}$ can cause neuroinflammation, and reduce spatial learning through the NF-κB p65/miR-574-5p/BACE1 molecular signalling axis (Ku et al., 2017). Long-term exposure to PM$_{2.5}$, the main component of diesel exhaust particles, results in impaired spatial memory and learning, increased proinflammatory cytokine levels in the TGE hippocampus, and an increased number of activated microglia in the dentate gyrus and CA1 region of the hippocampus (Ehsanifar et al., 2023). More interestingly, PM$_{2.5}$ also affects cognitive decline by causing sleep disorders.

Previous research on the damage to the nervous system caused by PM$_{2.5}$ is relatively thorough, while there are relatively few reports on nitrogen oxides and NO$_2$ (nitrogen dioxide) (Lam et al., 2016). The main sources of nitrogen oxides and NO$_2$, which are the main air pollutants, are automobile exhaust and the combustion of industrial fuels (Gholami et al., 2020; Cary and Ahmed, 2022). A cohort study of elderly people in the United States recorded daily NO$_2$ exposure and assessed cognitive function using the Chicago Cognitive Function Measurement (CCFM), and research has shown that an increase in long-term NO$_2$ exposure is associated with a significant decline in cognitive function (Tallon et al., 2017). In a large population-based study of French people, exposure to NO$_2$ was also found to be associated with poorer cognitive function, particularly regarding language skills and executive function (Zare Sakhvidi et al., 2022). As proven in animal experiments, increased NO levels in the mouse brain inhibit glutamate while promoting the GABAergic system (Tripathi et al., 2023). In addition, NO$_2$ has also been confirmed to damage mitochondrial energy metabolism in the brain (Yan et al., 2015).

According to our literature search, there are many discussions about the effects of PM$_{2.5}$, NO$_x$, and NO$_2$ on cognitive function, but there is a lack of animal experiments proving the relevant pathways influencing cognitive function. In addition, due to the interference of confounding factors, the causality determined in traditional observational studies has a certain degree of heterogeneity. However, clinical randomized controlled trials cannot be carried out due to ethical constraints. Whether air pollutants affect cognitive function has not been definitively determined. Mendelian randomization studies, in which germline genetic variants are used as instrumental variables to substitute for the exposure of interest, have been widely performed in recent years (Wang et al., 2022). Since genetic variation is already established at the time of zygote formation, the screened genetic variation data are related only to exposure, not to interference from confounding factors, and only affect the outcome through exposure, which can better infer causality (Bowden and Holmes, 2019). In this study, we rigorously screened instrumental variables to determine whether there was a causal relationship between PM$_{2.5}$, NO$_x$, and NO$_2$ and cognitive function.

2 METHODS

2.1 Data Sources

According to our previous literature, PM$_{2.5}$, NO$_x$, and NO$_2$ may have a certain relationship with cognitive function, and the genetic variation information for these three variables passed our strict screening. Both the exposure and outcome data were drawn from publicly available GWAS databases. Aggregate data for PM$_{2.5}$ (GWAS ID: ukb-b-10817), NO$_x$ (GWAS ID: ukb-b-12417), and NO$_2$ (GWAS ID: ukb-b-9942) were obtained from the UK Biobank, with 9,851,867 single nucleotide polymorphisms (SNPs) in each of the three datasets. Air pollution estimates for the year 2010 were modelled for each address using a land use regression (LUR) model developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) (http://www.escapeproject.eu), which was funded under the EU 7th Framework Programme. LUR model is a statistical regression model established by combining the relationship between atmospheric, soil and other pollutants...
and land use, traffic, industrial emissions, topography, climate, population density and other related factors to predict the pollutant concentration at any location in the study area. The mean values were 9.99 µg m⁻³ for PM₂.₅, 26.71 µg m⁻³ for NO₂, and 44.11 µg m⁻³ for NOₓ. The LUR model was based on the ESCAPE monitoring conducted between January 26, 2010, and January 18, 2011, and the air pollution estimates are representative of 2010.

The outcome data were also obtained from the UK Biobank, in which cognitive assessments were conducted via a computer touch-screen interface. Cognitive function is based on the brain receiving external information and actively guiding a person’s behaviour through processing, including memory, language, visuospatial function, execution, calculation, understanding and judgement (Kadic and Kurjak, 2018; Sakurai and Gamo, 2019). Fluid intelligence is the ability to solve problems and require logic and reasoning skills. The fluid intelligence score (Field 20016, GWAS ID: ukb-b-5238) is the number of times a participant answers the question correctly within two minutes. The reaction time test is a card game in which a button is pressed when two cards are the same. We measured reaction times to determine the correct match (field 20023, GWAS ID: ukb-b-16287). The pairing test is used to assess episodic visual memory by asking participants to remember the locations of matching cards, place the cards face down, and successfully pair the cards in as few attempts as possible. We used the number of incorrect matches in the round article (Field 399, GWAS ID: ukb-b-20498) for data analysis. The fluid intelligence score and pairing test have been tested and evaluated since 2012, and reaction times have been tested and evaluated since 2013.

2.2 Selection of Instrumental Variables

SNPs were utilized as instrumental variables (IVs) in MR analyses (Fig. 1). IVs should meet three assumptions: 1) there is a strong correlation between the IVs and exposure factors, 2) the IVs are

![Fig. 1. Principle of (A) MR and (B) flow chart.](image-url)
independent of confounding factors, and 3) the IVs can only affect the outcome through exposure factors (Sekula et al., 2016). We selected SNPs with a genome-wide significance level of \( p < 5 \times 10^{-8} \) as the IVs to guarantee that the selected SNPs were significantly associated with the relevant phenotype. In addition, to obtain independent SNPs, we set up strict clump windows (\( r^2 = 0.001, \text{kb} = 10,000 \) (Zhang et al., 2022; Sun et al., 2023)). Finally, we included 8 SNPs for each of the three pollutants.

### 2.3 Statistical Analysis

The TwoSample package article (version 0.5.7) of R software (version 4.3.1) was used for two-sample Mendelian randomization analysis. Inverse-variance weighted (IVW) models were used for the primary MR analysis to assess the causal relationship between exposures and outcomes. IVW analysis assumes that all genetic variants are valid instrumental variables and has strong causal detection power. Although we eliminated known confounding SNPs as much as possible, there may still be many unknown confounding factors that may have led to bias. Therefore, we also selected two other methods to test the reliability of the results, namely, MR-Egger regression and the weighted median method. The heterogeneity among IVs was tested through Cochrane’s Q-statistic calculation (Kennedy et al., 2020). At \( p < 0.05 \), the heterogeneity was considered significant. IVs were excluded one by one by the leave-one-out sensitivity test to judge the stability of the MR results. Since horizontal pleiotropy can lead to errors in results, we determined whether it existed through the intercept term of the MR-Egger model. After Bonferroni correction, \( p < 0.05/n \) (\( n = \) number of testing methods) was considered to indicate statistical significance.

### 3 RESULTS

#### 3.1 MR Analysis of PM2.5 and Cognitive Function

According to these three models, there was no causal relationship between the PM2.5 concentration and the fluid intelligence score, reaction time, or pairing (Table 1, Fig. 2). The Cochran Q test did not detect heterogeneity, and MR-Egger regression did not reveal horizontal pleiotropy (Table 2).

#### 3.2 MR Analysis of NOx and Cognitive Function

The IVW (\( \hat{\beta} = 0.879 \), 95% CI [–1.423, –0.336], \( p = 0.002 \)) and weighted median (\( \hat{\beta} = 0.847 \), 95% CI [–1.940, –0.204], \( p = 0.010 \)) models revealed a causal relationship between nitrogen oxides and fluid intelligence scores. There was no heterogeneity (\( Q = 11.34187, p = 0.124 \)) or pleiotropy (\( \text{intercept} = 0.006734, \text{se} = 0.020516, p = 0.754 \)), and a leave-one-out sensitivity test showed that our results were also reliable. No causal relationships were found between reaction time and pairing (Table 1, Fig. 2). However, Cochran’s Q test suggested heterogeneity (Table 2).

#### 3.3 MR Analysis of NOx and Cognitive Function

The weighted median model suggested a causal relationship between \( \text{NO}_{2} \) and pairing (\( \hat{\beta} = 0.204 \), 95% CI [0.039, 0.369], \( p = 0.015 \)). However, heterogeneity existed (Table 2). Horizontal

### Table 1. MR estimates of assessing the causal association between PM2.5, NOx, NO2, and cognitive function.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>NSNPs</th>
<th>MR-IVW (95% CI)</th>
<th>MR-Egger (95% CI)</th>
<th>MR-Weighted median (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PM2.5</td>
<td>Fluid intelligence score</td>
<td>8</td>
<td>–0.447(–0.979, 0.076)</td>
<td>0.099(–1.182, 0.045)</td>
<td>0.108(–1.172, 0.727)</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>8</td>
<td>0.121(–0.018, 0.260)</td>
<td>0.087(0.397, 0.667)</td>
<td>0.028(0.137, 0.296)</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>8</td>
<td>0.036(–0.111, 0.184)</td>
<td>0.629(0.235, 0.058)</td>
<td>0.229(0.034, 0.204)</td>
</tr>
<tr>
<td>NOx</td>
<td>Fluid intelligence score</td>
<td>8</td>
<td>–0.879(–1.423, –0.336)</td>
<td>0.002(–1.303, 0.292)</td>
<td>0.363(–1.847, –0.204)</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>8</td>
<td>0.104(–0.073, 0.280)</td>
<td>0.249(0.609, 1.348)</td>
<td>0.158(0.053, 0.235)</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>8</td>
<td>0.173(–0.179, 0.525)</td>
<td>0.335(0.169, 1.862)</td>
<td>0.851(0.212, 0.045)</td>
</tr>
<tr>
<td>NO2</td>
<td>Fluid intelligence score</td>
<td>8</td>
<td>–0.399(–1.108, 0.369)</td>
<td>0.348(0.125, 3.482)</td>
<td>0.944(–0.232, 0.408)</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>8</td>
<td>0.111(–0.119, 0.341)</td>
<td>0.344(0.155, 0.939)</td>
<td>0.790(0.204, 0.388)</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>8</td>
<td>0.166(–0.144, 0.477)</td>
<td>0.294(–0.672, 0.263)</td>
<td>0.354(0.204, 0.369)</td>
</tr>
</tbody>
</table>
**Fig. 2.** Scatter plots of causality. The slope of each line corresponding to the estimated MR effect in different models.
Table 2. Sensitivity analysis of MR analysis.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Outcome</th>
<th>Heterogeneity test</th>
<th>MR-Egger pleiotropy test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Q</td>
<td>P</td>
</tr>
<tr>
<td>PM$_{2.5}$</td>
<td>Fluid intelligence score</td>
<td>12.145</td>
<td>0.096</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>14.401</td>
<td>0.122</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>13.019</td>
<td>0.072</td>
</tr>
<tr>
<td>NO$_x$</td>
<td>Fluid intelligence score</td>
<td>11.342</td>
<td>0.124</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>16.374</td>
<td>0.022</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>65.928</td>
<td>9.79E-12</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>Fluid intelligence score</td>
<td>20.162</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>Reaction time</td>
<td>29.146</td>
<td>0.0001</td>
</tr>
<tr>
<td></td>
<td>Pairs matching</td>
<td>53.799</td>
<td>2.58E-09</td>
</tr>
</tbody>
</table>

Pleiotropy was not found by MR-Egger regression (Table 2). No causal relationship was found between NO$_2$ and fluid intelligence scores or reaction time (Table 1, Fig. 3).

4 DISCUSSION

To the best of our knowledge, this is the first study to use MR to establish a causal relationship between air pollutants and cognitive function. According to our findings, NO$_x$ affects fluid intelligence scores, suggesting some impairment of cognitive function.

An increasing number of epidemiological studies have focused on the impact of air pollution on cognitive function. Using data from the China Health and Retirement Longitudinal Study (CHARLS) collected from 2013 to 2015, environmental particulate pollutants were found to reduce cognitive function in older Chinese adults, especially women or individuals living in central China (Yao et al., 2022). Similarly, based on CHARLS data, research has shown that indoor air pollution caused by solid fuels can also reduce the mental state and situational memory of middle-aged and elderly people (Luo et al., 2021). A prospective cohort study in France also suggested that PM$_{2.5}$ is associated with a global decline in cognitive function (Duchesne et al., 2022). In multicentre birth cohorts in six European countries, exposure to PM$_{2.5}$ was found to be associated with lower fluid intelligence scores in children (Julvez et al., 2021). For Australian adolescents and adults, it was found that high NO$_2$ concentrations predicted poor executive function in both generations, high PM$_{2.5}$ concentrations predicted poor attention in adults, and reducing air pollution could improve cognitive function in adolescents, but not in adults (Wang et al., 2023). China has implemented a clean air program, which has led to significant declines in PM$_{3.5}$, PM$_{2.5}$, and NO$_2$ exposure, thus slowing the decline in cognitive function (Hu et al., 2022). A longitudinal cohort study of older women in American communities revealed that better air quality in later life was associated with a slower rate of cognitive decline (Younan et al., 2022). Improving air quality might improve cognitive function. Although our results were negative for PM$_{2.5}$ and NO$_2$, due to the heterogeneity of the results for NO$_2$, we may need to use other GWAS data to validate the results. This heterogeneity is likely due to genetic differences between individuals. At the same time, the weighted median method suggested that there was a causal relationship between NO$_2$ and pair matching, but the weighted median method may produce a robust causal effect with a large number of invalid instrumental variables; therefore, we cannot infer a causal relationship between NO$_2$ and cognitive function. In addition, MR genetic variants, although positively associated with exposure, usually have a small effect on most exposures, which leads to the risk of false-negative results (Larsson et al., 2023). At the same time, NO$_x$ mainly includes NO and NO$_2$. Whether NO$_x$ affects cognitive function is due to NO, the combined effect of NO and NO$_2$, or just because of different exposure concentrations needs to be further explored.

Air pollution can affect cognitive function through various mechanisms. Air pollution is believed to be associated with stroke, indicating that its impact on cognitive function may be indirect (Verhoeven et al., 2021). However, there are also studies indicating that air pollution can directly affect the nervous system through brain nutrient factors (Bos et al., 2014). Inflammation is also
believed to be involved in the pathogenesis of air pollutants (Arias-Pérez et al., 2020). There are also data indicating that PM$_{10}$, SO$_2$, and ozone are involved in the development of cognitive impairment (Schwela, 2000; Lee et al., 2020; Bello-Medina et al., 2022). However, additional animal experiments are needed to explore the specific mechanism by which air pollution affects cognitive function.

However, other studies have found no link between air pollution and cognitive function. In one area of Spain with high air pollution exposure, prenatal exposure to air pollution was not associated with cognitive or language function in children (Iglesias-Vázquez et al., 2022). A study based on data from the Rotterdam birth cohort in the Netherlands revealed that exposure to high levels of air pollution during pregnancy and childhood was not associated with cognitive decline in adolescents (Kusters et al., 2022). The association between air pollution exposure and cognitive function is very weak when considering confounding factors, according to a cohort study of the general population using the UK Biobank (Cullen et al., 2018). A possible reason for the different results may be the different populations studied. Most studies in older adults have shown that air pollution negatively affects cognition, which suggests the need for studies in different populations. Second, the exposure level and timing of air pollution exposure are not consistent among different
studies, which is also an important factor leading to different results. Moreover, observational studies are limited by a large number of confounding factors, and cognitive function is related to education level, sleep, exercise, etc. (Kolken et al., 2023; Ma et al., 2023a). This finding also reinforces the need for rigorous animal studies to confirm the relationship between air pollution and cognitive function.

Our study provides new evidence for the relationship between air pollution and cognitive function, suggesting that NOx reduces cognitive function, but there are still some limitations. First, the GWAS data are only from European populations, and genetic data for different regions, populations, and environments are lacking, so they cannot be extrapolated to other populations. We also need point system for the rest of the population. Second, although MR can minimize confounding factors, due to the complexity of reality, we cannot ensure the exclusion of all confounding factors, such as lifestyle habits, travel history, tests for contaminant interactions are lacking and so on. Fourth, exposure to air pollution may be either overestimated or underestimated because exposure to ambient air pollution at work is not available in the UK Biobank (Wang et al., 2021a). Finally, the number of SNPs we included was limited, which required us to rescreen instrumental variables or collect new GWAS data. For other air pollutants, because GWAS data were not available, Mendelian randomization studies could not be conducted. A large number of population-based cohort studies are needed to verify the effects of air pollution on cognitive function.

5 CONCLUSIONS

Our MR analysis showed that NOx lead to a decrease in fluid intelligence scores, indicating that it can impair cognitive function, especially logic and reasoning. For people living in polluted areas, early screening of cognitive function may prevent the occurrence of neurodegenerative diseases.

ACKNOWLEDGMENTS

The authors would like to thank the staff and participants of the UK Biobank for their meaningful contributions.

ADDITIONAL INFORMATION AND DECLARATIONS

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work has been supported by the Smart Accumulation and Talent Development Project of Xiangya Third Hospital, Central South University [grant number: YX202212].

Data Availability Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Supplementary Material

Supplementary material for this article can be found in the online version at https://doi.org/10.4209/aaqr.230326

REFERENCE


Julve, J., López-Vicente, M., Warembourg, C., Maitre, L., Philippat, C., Günzlow, K.B., Guxens, M., Evandt, J., Andrusaityte, S., Burgaleta, M., Casas, M., Chatzi, L., de Castro, M., Donaire-González,


