Effect of FOXO3 and Air Pollution on Cognitive Function: A Longitudinal Cohort Study of Older Adults in China From 2000 to 2014

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Abstract

Forkhead Box O 3 (FOXO3) genotype is strongly associated with human longevity and may be protective against neurodegeneration. Air pollution is a risk factor for cognitive decline and dementia. We aimed to study the individual and combined effects of FOXO3 and air pollution on cognitive function in a large prospective cohort with up to 14 years of follow-up. We measured cognitive function and impairment using the Mini-Mental State Examination (MMSE). We used tagging SNPs rs2253310, rs2802292, and rs4946936 to identify the FOXO3 gene, of which roughly half of the population had the longevity-associated polymorphism. We matched annual average fine particulate matter (PM2.5) concentrations within a 1 km² grid. We conducted cross-sectional and longitudinal analyses using multivariable linear and logistic regression models and generalized estimating equations. At baseline, carriers of the longevity-associated homozygous minor alleles of FOXO3 SNPs had a higher MMSE score than the carriers of homozygous major alleles. In the longitudinal follow-up, carriers of FOXO3 homozygous minor alleles had lower odds of cognitive impairment compared with noncarriers. Higher PM2.5 was associated with a lower MMSE score and higher odds of cognitive impairment. The positive effects of FOXO3 were the strongest in females, older people, and residents in areas with lower air pollution.

Keywords: Air pollution, Cognitive function, FOXO3, Gene–environment interaction
identified risk factors for dementia, accounting for 2.3% of 39.7% of the population attributable fraction (18). Air pollution is considered a late-life risk factor, affecting older adults, with a 75% risk factor prevalence (18). In developing countries such as China, a significant proportion of people experience high air pollution exposure, with estimates indicating roughly 15.5% (95% CI: 15.2%–15.9%) of older adults suffer from mild cognitive impairment (19). This number is expected to grow as the population ages.

In our study, we aimed to assess the associations between FOXO3 and cognitive function, as well as air pollution and cognitive function. We compared the genetic and environmental effects and assessed whether there were any interaction effects or effect modification by gender. To conduct this research, we utilized a longitudinal cohort study with 14 years of follow-up.

Method

Study Population
We used data from the Chinese Longitudinal Healthy Longevity Survey (CLHLS). The study included nationally representative regions covering 23 out of 31 provinces in China. The baseline survey started in 1998, and new participants were recruited to replace the deceased study participants during the follow-up surveys in 2000, 2002, 2005, 2008/2009, 2011/2012, and 2014. We included 9,231 participants aged 65 or older with genetic sequencing data and first interviewed in 2000, 2002, 2005, 2008/2009, and 2011/2012 (Figure S1) after excluding 291 without any PM2.5 measurement and 79 participants with missing baseline covariates data. Excluded participants were likely to be older and lived in rural regions (data not shown).

Cognitive Function Measurement
The CLHLS investigators used an adapted Chinese language version of the Mini-Mental State Examination (MMSE) as a measurement of cognitive function, with resurvey during follow-up to 2014. The scale is 0–30 points, a higher score indicating better cognitive function. We defined MMSE <24 score as having cognitive impairment according to a widely used criterion (20).

FOXO3 Genotype Ascertainment
Based on the results of CLHLS Genome-Wide Association Study (genotyping and quality control procedures can be found in a previous study) (21), the Beijing Genomics Institute carried out a replication study for 13,228 individuals using a well-designed and customized chip targeting 27,656 longevity-phenotype related single nucleotide polymorphisms (SNPs). We extracted the FOXO3 genotypic data from this replication study. The single SNP association analysis, genotype association analysis, linkage disequilibrium, and haplotype association analysis of CLHLS FOXO3 data were presented in a previous study (22). We used the tagging SNPs rs2253310, rs2802292, and rs4946936 to identify the FOXO3 gene as theirs (22). The minor/major alleles were T/C for rs4946936, G/T for rs2802292, and C/G for rs2253310, respectively.

Air Pollution Exposure Assessment
Ground-level PM2.5 concentrations were estimated by the Atmospheric Composition Analysis Group. They combined aerosol optical depth retrievals from the National Aeronautics and Space Administration’s Moderate Resolution Imaging Spectroradiometer, Multi-angle Imaging Spectro-Radiometer, and Sea-viewing Wide field-of-view Sensor satellite instruments; vertical profiles derived from the GEOS-Chem chemical transport model; and calibration to ground-based observations of PM2.5 using geographically weighted regression (23). The PM2.5 concentration estimates were highly consistent (R² = 0.81) with out-of-sample cross-validated PM2.5 concentrations from monitors (23). It was also found to be highly correlated to another exposure data set in China (24). Residential locations for each participant were collected via face-to-face household surveys. By linking residential locations to the nearest 1 km × 1 km PM2.5 grids, we could match the PM2.5 exposure for each participant. The annual PM2.5 was measured in the baseline year and in all the follow-up years for every participant.

Assessment of Covariates
We included the following baseline characteristics: age, gender, marital status, residence, education, smoking status, drinking status, and physical activity. We classified marital status into 2 categories: currently married and living with spouse as “married” and widowed/separated/divorced/never married/married but not living with spouse as “not married.” The survey used the Chinese residence classifications: village, town, and city. We further classified “City” and “Town” as urban areas and “Village” as rural areas. We used the schooling year to evaluate education level. We divided the regular exercise, smoking, and alcohol drinking status into 3 categories: “Current,” “Former,” and “Never.” For example, participants were asked “do you do exercise regularly at present (planned exercise like walking, playing balls, running, and so on)?” and/or “did you do exercise regularly in the past?”. We defined the regular exercise status as “Current” for participants who answered “Yes” to the first question, “Former” for those who answered “No” to the first question and “Yes” to the second question, and “Never” for those who answered “No” to both the questions.

Statistical Analysis
In the cross-sectional analyses, we examined the association between FOXO3 and MMSE score using linear regression model, FOXO3 and cognitive impairment using the logistic model. In the longitudinal analyses, we conducted the generalized estimating equations to test the association between FOXO3 and cognitive function. We further explored the interaction between FOXO3 and air pollution. We adjusted for age, sex, marriage, residence, education, exercise, smoking, and alcohol drinking. We set the nominal significance level at 0.05. We used R.4.0.3 to conduct all analyses.

Results

Population Characteristics
At baseline, the mean age was 82.5 (SD: 11.7), ranging from 65 to 112 for the total population. Females had the mean age of 84.6 ranging from 65 to 111, and males had the mean age of 80.1, ranging from 65 to 112, Females comprised 52.8% (n = 4,806) of the total population (Table 1). The baseline mean MMSE score was 24 (SD: 8), and 29.5% (n = 2,689) were considered to have cognitive impairment. The distributions of the 3 SNPs of FOXO3 were found to be similar across study population demographic characteristics, indicating Mendelian randomization. Surprisingly, PM2.5 exposure levels were quite similar between the urban and rural areas, probably due to peri-urbanization processes and geographical expansion of industries to smaller cities. The average baseline MMSE scores were roughly even by FOXO3 SNPs and air pollution exposure (Table 1 and Supplementary Table 1).
Table 1. Baseline Population Characteristics

| Variable | rs2802292 (n = 4,574) | TG (n = 3,777) | MMSE Score: Mean (SD) | Overall (n = 9,102) |
|----------|------------------------|----------------|------------------------|----------------------|
| Sex      | Male                   | 2,634 (57.6)  | 2,634 (57.6)           | 2,634 (57.6)         |
|          | Female                 | 2,100 (52.4)  | 2,100 (52.4)           | 2,100 (52.4)         |
| Age      | Mean (SD)              | 62.0 (13.1)   | 62.0 (13.1)            | 62.0 (13.1)          |
|          | Median [Min, Max]      | 60.0 (12.0)   | 60.0 (12.0)            | 60.0 (12.0)          |
| Education | Mean (SD)              | 6.3 (1.3)     | 6.3 (1.3)              | 6.3 (1.3)            |
|          | Median [Min, Max]      | 5.0 (0.0)     | 5.0 (0.0)              | 5.0 (0.0)            |
| Residence| Mean (SD)              | 93.0 (27.4)   | 93.0 (27.4)            | 93.0 (27.4)          |
|          | Median [Min, Max]      | 90.0 (0.0)    | 90.0 (0.0)             | 90.0 (0.0)           |
| Marital  | Mean (SD)              | 93.0 (27.4)   | 93.0 (27.4)            | 93.0 (27.4)          |
|          | Median [Min, Max]      | 90.0 (0.0)    | 90.0 (0.0)             | 90.0 (0.0)           |
| Exercise | Mean (SD)              | 93.0 (27.4)   | 93.0 (27.4)            | 93.0 (27.4)          |
|          | Median [Min, Max]      | 90.0 (0.0)    | 90.0 (0.0)             | 90.0 (0.0)           |
| Smoking  | Mean (SD)              | 93.0 (27.4)   | 93.0 (27.4)            | 93.0 (27.4)          |
|          | Median [Min, Max]      | 90.0 (0.0)    | 90.0 (0.0)             | 90.0 (0.0)           |
| Alcohol  | Mean (SD)              | 93.0 (27.4)   | 93.0 (27.4)            | 93.0 (27.4)          |
|          | Median [Min, Max]      | 90.0 (0.0)    | 90.0 (0.0)             | 90.0 (0.0)           |
| rs2253310| Mean (SD)              | 47.0 (2.0)    | 47.0 (2.0)             | 47.0 (2.0)           |
|          | Median [Min, Max]      | 47.0 (2.0)    | 47.0 (2.0)             | 47.0 (2.0)           |
| rs4946396| Mean (SD)              | 3.0 (0.0)     | 3.0 (0.0)              | 3.0 (0.0)            |
|          | Median [Min, Max]      | 3.0 (0.0)     | 3.0 (0.0)              | 3.0 (0.0)            |
| rs4946396| Mean (SD)              | 4.0 (0.0)     | 4.0 (0.0)              | 4.0 (0.0)            |
|          | Median [Min, Max]      | 4.0 (0.0)     | 4.0 (0.0)              | 4.0 (0.0)            |
| rs280292 | Mean (SD)              | 4.0 (0.0)     | 4.0 (0.0)              | 4.0 (0.0)            |
|          | Median [Min, Max]      | 4.0 (0.0)     | 4.0 (0.0)              | 4.0 (0.0)            |

Notes: SD = standard deviation; MMSE = Mini-Mental State Examination; PM 2.5 = Fine particulate matter.
The Association Among FOXO3, PM2.5, and Cognitive Function at Baseline

Higher PM2.5 was associated with lower MMSE score (each 10 μg/m³ increase of PM2.5: −0.39, 95% confidence interval [CI]: −0.50, −0.28; Table 2) and higher odds of cognitive impairment (odds ratio [OR] for each 10 μg/m³ increase of PM2.5: 1.09 [95% CI: 1.04, 1.13]; Supplementary Table 2). Homozygous minor alleles of FOXO3 SNPs had higher MMSE score than the homozygous major alleles (mean difference of MMSE score [95% CI]: 0.51 [−0.08, 1.09] for rs4946936, 0.65 [0.12, 1.17] for rs2802292, 0.59 [0.07, 1.11] for rs2253310; Table 2). But the negative associations between FOXO3 SNPs and cognitive impairment were not statistically significant (Supplementary Table 2). These associations between FOXO3 and MMSE scores were attenuated after adjusting for PM2.5 (Table 2).

The Association Among FOXO3, PM2.5, and Repeatedly Measured Cognitive Function

In the longitudinal analyses, each 10 μg/m³ increase of PM2.5 was associated with a lower MMSE score (mean difference: −0.20 [95% CI: −0.28, −0.13]; Table 3) and slightly higher odds of cognitive impairment (OR: 1.04 [95% CI: 1.01, 1.07]; Supplementary Table 3). Homozygous minor alleles of FOXO3 SNPs had higher MMSE score than the homozygous major alleles (mean difference of MMSE score [95% CI]: 0.48 [0.05, 0.90] for rs4946936, 0.54 [0.16, 0.92] for rs2802292, 0.54 [0.16, 0.93] for rs2253310; Table 3). The odds difference of cognitive impairment was not statistically significant among the different genotypes (Table 3 and Supplementary Table 3). These associations were also attenuated by adjusting for PM2.5.

We also identified significant interactions between FOXO3 SNPs and PM2.5 on the repeatedly measured MMSE score. The negative association between PM2.5 and MMSE score was more evident among the participants with homozygous minor alleles, while the positive association between homozygous minor alleles and MMSE score declined with the increase of PM2.5 exposure (interaction term for homozygous minor alleles and PM2.5 was negative, p value <.05; Table 3). As shown in Figure 1 of the predicted MMSE score based on the interaction model, homozygous minor alleles of rs2802292 had a higher predicted MMSE score than homozygous major alleles under low PM2.5 exposure, not under high PM2.5 exposure. Participants under the high PM2.5 exposure had lower MMSE scores than those under the low PM2.5 exposure, and the difference of the predicted MMSE score between low and high PM2.5 exposure was the most significant among those with homozygous major alleles (Figure 2). In the stratified analyses, the protective effect of FOXO3 SNPs on cognitive function also only existed under low PM2.5 exposure (PM2.5 ≤50 μg/m³), but disappeared under high PM2.5 exposure (Table 4). The effect of PM2.5 was also stronger for homozygous minor alleles than homozygous major alleles of FOXO3 (Supplementary Table 4).

We found an interaction between the FOXO3 SNPs and age, education, marriage, and drinking alcohol, no significant interaction between sex, residence, exercise, and smoking. In the stratified analyses, there was a positive association between FOXO3 homozygous minor allele and MMSE score in the female or participants aged 80 or older, but it did not show in the male or participants aged younger than 80 (Table 5 and Supplementary Table 5). The negative association between PM2.5 and MMSE score was stronger in the female than the male, but reversed in participants aged younger than 80 (Table 5 and Supplementary Table 5).

Discussion

We found independent as well as interactive effects of FOXO3 and PM2.5 exposure. Carriers of homozygous minor alleles of FOXO3 SNPs were protected against cognitive decline. Simultaneously, we documented a harmful association between PM2.5 exposure and cognitive function. Moreover, we identified a significant gene–environment interaction between FOXO3 and PM2.5 on cognitive function. The effect modification analyses yielded insightful findings. In the gender-specific analyses, we found the effect of FOXO3 to be evident only in females, but not in males, with statistical significance for rs2802292 and rs2253310. The detrimental effect of PM2.5 exposure is visible for both genders. However, female participants experienced more than twice the detrimental effect for a 10 μg/m³ increase in air pollution (~0.26 points in females compared with ~0.12 points in males). Comparing effect sizes, the beneficial effect of carrying homozygous minor alleles for FOXO3 is equivalent to about twice the detrimental effect of 10 μg/m³ PM2.5 (0.47 points for rs2802292 vs −0.20 points for each 10 μg/m³ increase of PM2.5). Interestingly, the protective effect of FOXO3 homozygous minor allele carriers was only evidenced in participants living in areas of low air pollution. In our age-stratified analysis, we see the protective effect of FOXO3 tended to be higher in those with advanced age. The older population also appeared to be affected more by air pollution than the younger ones. Our cohort contained a large proportion of those older than the age of 80 years. As population demographics are shifting toward a longer life expectancy, our findings are informative for a better understanding of dementia.

Previous studies in invertebrates and mammals have related FoxO with neurological outcomes and cognitive ability (10–13). In human populations, the role of FOXO3 SNPs in longevity has been repeatedly documented (3,17,25–27); however, evidence of the role of FOXO3 with regard to cognitive function remains scarce. FOXO3 SNPs were initially reported to be associated with longevity and healthy aging in a male American of Japanese ancestry population. The longevity cases had a higher prevalence of FOXO3 minor allele and similar levels of cognitive function despite being more than a decade older than controls (17). A study investigated the association of 15 FOXO3 SNPs with aging-related traits including cognitive function in 1,088 Danish oldest-old individuals and only found associations of FOXO3 with activities of daily living and bone fracture (28). We identified a significant association between FOXO3 SNPs and cognitive function in a larger longitudinal cohort of older Chinese adults. The possible pathway could be FOXO3 acts through the targeted genes, regulating a wide range of neuronal functions that critically modulate neuronal development and neurodegenerative diseases, including neurogenesis and neuronal regeneration, apoptosis, and oxidative stress (16). Animal studies also indicate FOXO3 phosphorylation was lower in females than in males and was associated with higher levels of protein ubiquitination, yielding one possible explanation of sex difference in our findings (29). Furthermore, there is an incomplete understanding of the multifaceted driving forces behind the gender differences in life expectancy between women and men. Environmental and genetic factors can be simultaneously at play, and the gender effect modification on longevity needs to be more thoroughly explored.

A prior systematic review of at least 13 longitudinal cohort studies found air pollutants, particularly PM2.5 exposure to be associated with incident dementia (30). These findings are supported by animal models of air pollution exposure and neurodegenerative outcome measures. Documented mechanistic pathways include...
Table 2. Associations of FOXO3 SNPs, PM2.5, and MMSE Score at Baseline (Cross-sectional Analyses)

| Term                          | Model With PM2.5 |            | Model With FOXO |            | Model With PM2.5 and FOXO |            | Model With PM2.5, FOXO, and Interaction Term |            |
|-------------------------------|------------------|------------|-----------------|------------|---------------------------|------------|----------------------------------------------|------------|
|                               | Predicted Change in MMSE Score (95% CI) | p | n | Predicted Change in MMSE Score (95% CI) | p | Predicted Change in MMSE Score (95% CI) | p | Predicted Change in MMSE Score (SE) | p |
| rs4946936 (CC as reference)   |                  | 4.00       | 3.625           | 0.13 (−0.16, 0.42) | 0.389 | 0.07 (−0.22, 0.36) | 0.648 | −0.39 (0.60) | 0.51 |
| TC                            |                  | 577        | 0.51 (−0.08, 1.09) | 0.090 | 0.38 (−0.20, 0.96) | 0.202 | −0.38 (−0.49, −0.27) | <0.01 |
| PM2.5 in 10 μg/m³              | −0.39 (−0.5, −0.28) | 0.090     | 0.09 (0.11) | 0.242 |
| rs4946936 TC × PM2.5           |                  | 4574       | 0.17 (−0.12, 0.46) | 0.256 | 0.14 (−0.15, 0.43) | 0.358 | −0.10 (0.60) | 0.093 |
| rs4946936 TT × PM2.5           |                  | 3777       | 0.65 (0.12, 1.17) | 0.015 | 0.53 (0.01, 1.05) | 0.048 | 1.48 (1.02) | 0.145 |
| rs2802292 (TT as reference)   |                  | 751        | −0.38 (−0.49, −0.27) | <0.01 | −0.43 (0.08) | <0.01 |
| TG                            |                  | 3747       | 0.15 (−0.14, 0.45) | 0.298 | 0.12 (−0.17, 0.41) | 0.407 | −0.82 (0.60) | 0.17 |
| GG                            |                  | 746        | 0.59 (0.07, 1.11) | 0.027 | 0.47 (−0.05, 0.90) | 0.076 | 1.59 (1.02) | 0.119 |
| PM2.5 in 10 μg/m³              | −0.39 (−0.5, −0.28) | 0.19 (0.11) | 0.103 |
| rs2802292 GC × PM2.5           |                  | 4609       | −0.38 (−0.49, −0.27) | <0.01 | −0.44 (0.08) | <0.01 |
| rs2802292 CC × PM2.5           |                  | 347       | 0.19 (0.11) | 0.103 |
| rs2253310 (GG as reference)   |                  | 4609       | −0.39 (−0.5, −0.28) | <0.01 | −0.23 (0.20) | 0.244 |

Notes: MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter; SNP = single nucleotide polymorphism. All the above linear models adjusted for age, sex, ethnicity, education, residence, marriage, exercise, smoking, and drinking alcohol. Beta was the coefficient estimation of the corresponding variable in the model, indicating the mean difference of the MMSE score between the comparison groups of the categorical variable or for each unit increment in the continuous variable.
cerebrovascular and cardiovascular harm, β amyloid formation, and accumulation of tau protein and their precursors (31–33). Nonetheless, the relationship between PM 2.5 and dementia is still subject to further study to control for potential residual confounding.

In a prior study using the same cohort, investigators found PM2.5 to be associated with a higher risk of cognitive impairment (MMSE <18) in CLHLS using the Cox model (HR: 1.051; 95% CI: 1.023, 1.079) (34). While changes in MMSE score are not monotonical over follow-up, we found that higher PM2.5 exposure was associated with lower MMSE score over time and higher odds of cognitive impairment.

Our study has several strengths. First, this is a novel research hypothesis on the interaction of FOXO3 SNPs and air pollution on cognitive function. Second, we used a large longitudinal cohort to measure changes in cognitive function and ambient air pollution during the follow-up. Third, our study covered a vast geographic area, which allowed us to test for effect modification and dose–response relationships. We also recognize several limitations of our study.

Table 3. Association of FOXO3 SNPs, PM2.5, and MMSE Score (Longitudinal Analysis)

| Term | Model—PM2.5 | Model—FOXO | Model—PM2.5 + FOXO | Model—PM2.5 × FOXO |
|------|-------------|------------|--------------------|-------------------|
| rs4946936 (CC as reference) | Beta (95% CI) | p | Beta (95% CI) | p | Beta (95% CI) | p | Beta (SE) | p |
| TC | -0.04 (-0.25, 0.18) | .739 | -0.07 (-0.29, 0.14) | .510 | -0.07 (0.43) | .869 |
| TT | 0.48 (0.05, 0.90) | .029 | 0.40 (-0.02, 0.83) | .063 | 2.39 (0.77) | .002 |
| PM2.5 in 10 μg/m³ | -0.20 (-0.28, -0.13) | <.001 | -0.20 (-0.28, -0.12) | <.001 | -0.18 (0.05) | .001 |
| rs4946936 TC × PM2.5 in 10 μg/m³ | 0 (0.08) | .993 |
| rs4946936 TT × PM2.5 in 10 μg/m³ | 0.42 (0.16) | .009 |
| rs2802292 (TT as reference) | | | | |
| TG | -0.03 (-0.25, 0.18) | .761 | -0.05 (-0.27, 0.16) | .632 | -0.50 (0.43) | .245 |
| GG | 0.54 (0.16, 0.92) | .006 | 0.47 (0.09, 0.86) | .015 | 2.28 (0.68) | .001 |
| PM2.5 in 10 μg/m³ | -0.20 (-0.28, -0.13) | <.001 | -0.20 (-0.27, -0.12) | <.001 | -0.20 (0.05) | <.001 |
| rs2802292 TG × PM2.5 in 10 μg/m³ | 0.09 (0.08) | .271 |
| rs2802292 GG × PM2.5 in 10 μg/m³ | -0.38 (0.14) | .006 |
| rs2253310 (GG as reference) | | | | |
| GC | 0 (-0.21, 0.22) | .988 | -0.02 (-0.23, 0.20) | .871 | -0.35 (0.43) | .409 |
| CC | 0.54 (0.16, 0.93) | .006 | 0.48 (0.09, 0.86) | .015 | 2.41 (0.68) | <.001 |
| PM2.5 in 10 μg/m³ | -0.20 (-0.28, -0.13) | <.001 | -0.20 (-0.27, -0.12) | <.001 | -0.19 (0.05) | <.001 |
| rs2253310 GC × PM2.5 in 10 μg/m³ | 0.07 (0.08) | .405 |
| rs2253310 CC × PM2.5 in 10 μg/m³ | -0.41 (0.14) | .003 |

Notes: MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter; SNP = single nucleotide polymorphism. All the above generalized estimate equation linear models adjusted for age, sex, ethnicity, education, residence, marriage, exercise, smoking, and drinking alcohol. Beta was the coefficient estimation of the corresponding variable in the model, indicating the mean difference of the MMSE score between the comparison groups of the categorical variable or for each unit increment in the continuous variable.

Figure 1. The predicted MMSE score (95% CI) of different genotypes of rs2802292 at different PM2.5 levels. Notes: The predicted MMSE score was calculated based on the generalized estimating equation with the interaction term of rs2802292 and PM2.5, adjusting for age, sex, education, residence, marriage, exercise, smoking, and drinking alcohol. The PM2.5 level of 3.70, 5.03, and 6.37 were the mean – SD, mean, and mean + SD of PM2.5 (10 μg/m³). MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter.

Figure 2. The predicted MMSE score (95% CI) of different PM2.5 exposure levels for different genotypes of rs2802292. Notes: The predicted MMSE score was calculated based on the generalized estimating equation with the interaction term of rs2802292 and PM2.5 group, adjusting for age, sex, education, residence, marriage, exercise, smoking, and drinking alcohol. The cutoff point for the low and high exposure of PM2.5 was 50 μg/m³. MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter.
observational cohort study. First, we could not elucidate the mechanistic etiology of FOXO3 SNPs interacting with PM$_2.5$ on neurological health at the biological, epigenetic, and molecular levels. Second, we did not have the personalized exposure data for air pollution and relied on ambient PM$_2.5$ levels. We cannot be certain that there was no healthy-worker survivor bias where healthier participants may be exposed to higher levels of air pollution because of occupation or location. Despite this possibility, we still found a robust association between ambient PM$_2.5$ exposure and cognitive function decline. Third, we utilized the MMSE to measure cognitive function, while considered a good proxy and used extensively in clinical and research settings to measure cognitive impairment, it is nonetheless not a clinical diagnosis of dementia or any other nosological entity. Fourth, like all longitudinal cohorts, we have informative censoring with 24% of participants only having baseline data due to mortality or loss of follow-up in the subsequent interview. Furthermore, our cohort of the Chinese population may limit the finding’s generalizability to other populations, but our research contributes to the reproducibility of prior analyses in European populations. A recurrent question in observational studies is the residual confounding. Our study adds to numerous previous cohort findings on the association between air pollution exposure and dementia. Our study is the first to examine air pollution and cognitive function as an exposure–outcome pair, while taking FOXO3 genotype into account. With FOXO3 exhibiting Mendelian randomization, it allowed us to use the genetic variant as an instrumental variable in order to infer a causal link.

In conclusion, our study demonstrated a protective effect of FOXO3 on cognitive function among older adults, confirming prior findings with regard to the important role of FoxO proteins in the neurological system (35,36). The positive impact of FOXO3 appeared to be higher in older people, females, and among residents in places of low-level air pollution exposure. This population health (epidemiologic) finding supplements biological research on gene–environment interaction in elucidating and potentially improving the health span of the brain and nervous system, both of which are vitally important for healthy aging and longevity.

| Table 4. Association Between FOXO3 and MMSE Score Stratified by High and Low PM$_{2.5}$ Concentration (Longitudinal Analysis) |
|---------------------------------------------------------------|
| PM$_{2.5}$ ≤50 μg/m$^3$ | PM$_{2.5}$ >50 μg/m$^3$ |
| Term | Participants | Predicted Change in MMSE Score (95% CI) | $p$ | Participants | Predicted Change in MMSE Score (95% CI) | $p$ |
| rs4946936 | | | | | | |
| CC | 2 040 | Reference | — | 2 860 | Reference | — |
| TC | 1 810 | -0.11 (-0.41, 0.2) | .489 | 1 815 | -0.01 (-0.3, 0.29) | .956 |
| TT | 320 | 0.81 (0.29, 1.33) | .002 | 257 | -0.06 (-0.75, 0.63) | .861 |
| rs2802292 | | | | | | |
| TT | 1 936 | Reference | — | 2 638 | Reference | — |
| TG | 1 820 | -0.2 (-0.5, 0.11) | .214 | 1 957 | 0.11 (-0.19, 0.4) | .475 |
| GG | 414 | 0.81 (0.33, 1.29) | .001 | 337 | 0.08 (-0.51, 0.66) | .794 |
| rs2253310 | | | | | | |
| GG | 1 950 | Reference | — | 2 659 | Reference | — |
| GC | 1 812 | -0.13 (-0.44, 0.18) | .404 | 1 935 | 0.11 (-0.18, 0.4) | .464 |
| CC | 408 | 0.84 (0.35, 1.32) | .001 | 338 | 0.06 (-0.35, 0.65) | .843 |

Notes: MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter. All the above generalized estimate equation models adjusted for age, sex, ethnicity, education, residence, marriage, exercise, smoking, and drinking alcohol. These models were built for each SNP separately.

| Table 5. Association of FOXO3, PM$_{2.5}$, and MMSE Score Stratified by Gender (Longitudinal Analysis) |
|---------------------------------------------------------------|
| Male | Female |
| Term | Participants | Predicted Change in MMSE Score (95% CI) | $p$ | Participants | Predicted Change in MMSE Score (95% CI) | $p$ |
| rs4946936 | | | | | | |
| CC | 2 367 | Reference | — | 2 533 | Reference | — |
| TC | 1 673 | 0.16 (-0.13, 0.44) | .278 | 1 952 | -0.19 (-0.51, 0.14) | .260 |
| TT | 256 | 0.22 (-0.37, 0.81) | .463 | 321 | 0.63 (0.03, 1.26) | .035 |
| rs2802292 | | | | | | |
| TT | 2 205 | Reference | — | 2 369 | Reference | — |
| TG | 1 747 | 0.11 (-0.17, 0.39) | .432 | 2 030 | -0.14 (-0.47, 0.18) | .383 |
| GG | 344 | 0.28 (-0.25, 0.81) | .304 | 407 | 0.72 (0.17, 1.26) | .010 |
| rs2253310 | | | | | | |
| GG | 2 227 | Reference | — | 2 382 | Reference | — |
| GC | 1 726 | 0.17 (-0.1, 0.45) | .222 | 2 021 | -0.13 (-0.46, 0.19) | .420 |
| CC | 343 | 0.28 (-0.25, 0.81) | .297 | 403 | 0.71 (0.16, 1.26) | .011 |
| PM$_{2.5}$ per 10 μg/m$^3$ | 4 296 | -0.12 (-0.22, -0.02) | .016 | 4 806 | -0.26 (-0.38, -0.15) | <.001 |

Notes: MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter. All the above generalized estimating equation models adjusted for age, ethnicity, education, residence, marriage, exercise, smoking, and drinking alcohol. The model was built for each SNP separately.
Supplementary Material

Supplementary data are available at The Journals of Gerontology, Series A: Biological Sciences and Medical Sciences online.

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Conflict of Interest

None declared.

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