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Special Issue: The FOXO3 Gene and Its Relation to Lifespan and Healthspan

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 ($PM_{2.5}$) concentrations within a 1 km^2 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 $PM_{2.5}$ 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

Forkhead/winged helix box gene group O3 (FoxO3) proteins are among a set of evolutionarily conserved transcription factors at a central integration hub for many important cellular functions that have exhibited consistent association with longevity in many diverse species, from hydra to humans (1–6). As downstream regulators of insulin/insulin-like growth factor (IGF-1) signaling, FoxO transcription factors trigger a variety of cellular processes by regulating target genes, which affect organismal processes including tumor suppression, development, metabolism, and longevity in mammals (7–9). Moreover, evidence is accumulating for an important role of FoxO3 in the prevention of age-related diseases, including neurodegenerative diseases, through the promotion of neuronal health, autophagy, and the downregulation of oxidative stress, among other mechanisms (9–12). These neuronal functions of FoxO transcription factors are conserved in many organisms ranging from *Caenorhabditis elegans* and *Drosophila* to mammals (10). Studies have also indicated regulatory roles for FoxO proteins in the pathogenesis, diagnosis, and potential treatment of Alzheimer's disease (13–16). The longevity-associated minor alleles of *FOXO3* have also been linked to high cognitive function in a population study of American men of Japanese ancestry (17). However, such population studies in human beings are rare and there is a need to expand studies of the relationship between *FOXO3* and cognitive decline in other populations. In particular, longitudinal cohorts can also shed light on relative effect sizes of genetic and environmental interactions, such as air pollution.

According to the recent Lancet Commission on Dementia Prevention, Intervention and Care, air pollution is one of the 12

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 $PM_{2.5}$ 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 $PM_{2.5}$ 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 $PM_{2.5}$ using geographically weighted regression (23). The $PM_{2.5}$ concentration estimates were highly consistent (R^2 = 0.81) with out-of-sample cross-validated $PM_{2.5}$ 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 $PM_{2.5}$ grids, we could match the $PM_{2.5}$ exposure for each participant. The annual $PM_{2.5}$ 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, $PM_{2.5}$ 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

	rs2802292				$PM_{2.5}$ in 10 μg/m³			MMSE Score: Mean (*SD*)	Overall
Variable	TT (*n*: 4 574)	TG (*n*: 3 777)	GG (*n*: 751)	*p*	<5 (*n*: 4 170)	≥5 (*n*: 4 932)	*p*		(*n*: 9 102)
MMSE score									
Mean (*SD*)	23.8 (8.3)	23.8 (8.2)	24.2 (7.7)	.326	24.3 (7.5)	23.3 (8.8)	<.001	—	23.8 (8.2)
Median [Min, Max]	27.0 [0, 30.0]	27.0 [0, 30.0]	27.0 [0, 30.0]		27.0 [0, 30.0]	27.0 [0, 30.0]		—	27.0 [0, 30.0]
Cognitive impairment: n (%)									
Yes	1 339 (29.3)	1 132 (30.0)	218 (29.0)	.746	1 154 (27.7)	1 535 (31.1)	<.001	13.5 (8.4)	2 689 (29.5)
No	3 235 (70.7)	2 645 (70.0)	533 (71.0)		3 016 (72.3)	3 397 (68.9)		28.1 (1.8)	6 413 (70.5)
$PM_{2.5}$ in 10 μg/m³									
Mean (*SD*)	5.2 (1.3)	5.1 (1.3)	4.8 (1.3)	<.001	4.0 (0.8)	6.1 (0.8)	<.001	—	5.1 (1.3)
Median [Min, Max]	5.3 [1.2, 10.8]	5.1 [0.7, 10.8]	4.8 [2.1, 8.4]		4.2 [0.7, 5.0]	6.0 [5.0, 10.8]		—	5.2 [0.7, 10.8]
$PM_{2.5}$ in 10 μg/m³: n (%)									
<5	1 936 (42.3)	1 820 (48.2)	414 (55.1)	<.001	—	—		24.3 (7.5)	4 170 (45.8)
≥5	2 638 (57.7)	1 957 (51.8)	337 (44.9)		—	—		23.3 (8.8)	4 932 (54.2)
Sex: n (%)									
Male	2 205 (48.2)	1 747 (46.3)	344 (45.8)	.149	1 947 (46.7)	2 349 (47.6)	.384	25.8 (6.8)	4 296 (47.2)
Female	2 369 (51.8)	2 030 (53.7)	407 (54.2)		2 223 (53.3)	2 583 (52.4)		22 (9)	4 806 (52.8)
Age									
Mean (*SD*)	82.3 (11.7)	82.7 (11.8)	82.7 (11.4)	.239	83.0 (11.4)	82.1 (12.0)	<.001	—	82.5 (11.7)
Median [Min, Max]	82.0 [65.0, 112.0]	83.0 [65.0, 111.0]	83.0 [65.0, 107.0]		84.0 [65.0, 112.0]	81.0 [65.0, 110.0]		—	83.0 [65.0, 112.0]
Ethnicity									
Han	4 325 (94.6)	3 483 (92.2)	684 (91.1)	<.001	3 612 (86.6)	4 880 (98.9)	<.001	23.7 (8.3)	8 492 (93.3)
Other	249 (5.4)	294 (7.8)	67 (8.9)		558 (13.4)	52 (1.1)		25.1 (7.3)	610 (6.7)
Education year: n (%)									
0 year	2 625 (57.4)	2 220 (58.8)	427 (56.9)	.676	2 358 (56.5)	2 914 (59.1)	.00197	21.6 (9.1)	5 272 (57.9)
1~6 years	1 425 (31.2)	1 143 (30.3)	233 (31.0)		1 360 (32.6)	1 441 (29.2)		26.5 (5.9)	2 801 (30.8)
≥6 years	524 (11.5)	414 (11.0)	91 (12.1)		452 (10.8)	577 (11.7)		27.7 (5)	1 029 (11.3)
Residence: n (%)									
Urban	1 669 (36.5)	1 362 (36.1)	256 (34.1)	.445	1 654 (39.7)	1 633 (33.1)	<.001	24.6 (7.6)	3 287 (36.1)
Rural	2 905 (63.5)	2 415 (63.9)	495 (65.9)		2 516 (60.3)	3 299 (66.9)		23.3 (8.5)	5 815 (63.9)
Marriage: n (%)									
Married	1 940 (42.4)	1 510 (40.0)	297 (39.5)	.051	1 571 (37.7)	2 176 (44.1)	<.001	26.9 (5)	3 747 (41.2)
Not married	2 634 (57.6)	2 267 (60.0)	454 (60.5)		2 599 (62.3)	2 756 (55.9)		21.6 (9.3)	5 355 (58.8)
Exercise: n (%)									
Current	1 451 (31.7)	1 166 (30.9)	219 (29.2)	.606	1 418 (34.0)	1 418 (28.8)	<.001	26.1 (6.1)	2 836 (31.2)
Former	285 (6.2)	231 (6.1)	52 (6.9)		330 (7.9)	238 (4.8)		21.3 (9.5)	568 (6.2)
Never	2 838 (62.0)	2 380 (63.0)	480 (63.9)		2 422 (58.1)	3 276 (66.4)		22.9 (8.8)	5 698 (62.6)
Smoking: n (%)									
Current	1 020 (22.3)	814 (21.6)	145 (19.3)	.123	907 (21.8)	1 072 (21.7)	.547	26 (6.3)	1 979 (21.7)
Former	628 (13.7)	514 (13.6)	88 (11.7)		546 (13.1)	684 (13.9)		24.7 (7.7)	1 230 (13.5)
Never	2 926 (64.0)	2 449 (64.8)	518 (69.0)		2 717 (65.2)	3 176 (64.4)		22.9 (8.7)	5 893 (64.7)
Alcohol: n (%)									
Current	1 036 (22.6)	806 (21.3)	144 (19.2)	.00494	901 (21.6)	1 085 (22.0)	.0896	25.3 (7.1)	1 986 (21.8)
Former	451 (9.9)	359 (9.5)	51 (6.8)		425 (10.2)	436 (8.8)		24.3 (7.4)	861 (9.5)
Never	3 087 (67.5)	2 612 (69.2)	556 (74.0)		2 844 (68.2)	3 411 (69.2)		23.2 (8.6)	6 255 (68.7)
rs2253310: n (%)									
GG	4 562 (99.7)	47 (1.2)	0 (0)	<.001	1 950 (46.8)	2 659 (53.9)	<.001	23.8 (8.3)	4 609 (50.6)
GC	12 (0.3)	3 727 (98.7)	8 (1.1)		1 812 (43.5)	1 935 (39.2)		23.8 (8.2)	3 747 (41.2)
CC	0 (0)	3 (0.1)	743 (98.9)		408 (9.8)	338 (6.9)		24.2 (7.8)	746 (8.2)
rs4946936: n (%)									
CC	4 444 (97.2)	450 (11.9)	6 (0.8)	<.001	2 040 (48.9)	2 860 (58.0)	<.001	23.8 (8.3)	4 900 (53.8)
TC	128 (2.8)	3 285 (87.0)	212 (28.2)		1 810 (43.4)	1 815 (36.8)		23.8 (8.1)	3 625 (39.8)
TT	2 (0.0)	42 (1.1)	533 (71.0)		320 (7.7)	257 (5.2)		23.9 (8.1)	577 (6.3)
rs2802292: n (%)									
TT	—	—	—		1 936 (46.4)	2 638 (53.5)	<.001	23.8 (8.3)	4 574 (50.3)
TG	—	—	—		1 820 (43.6)	1 957 (39.7)		23.8 (8.2)	3 777 (41.5)
GG	—	—	—		414 (9.9)	337 (6.8)		24.2 (7.7)	751 (8.3)

Notes: SD = standard deviation; MMSE = Mini-Mental State Examination; PM 2.5 = Fine particulate matter.

The Association Among *FOXO3*, $PM_{2.5}$, and Cognitive Function at Baseline

Higher $PM_{2.5}$ was associated with lower MMSE score (each 10 μg/m³ increase of $PM_{2.5}$: −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 $PM_{2.5}$: 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 $PM_{2.5}$ (Table 2).

The Association Among *FOXO3*, $PM_{2.5}$, and Repeatedly Measured Cognitive Function

In the longitudinal analyses, each 10 μg/m³ increase of $PM_{2.5}$ 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 $PM_{2.5}$.

We also identified significant interactions between *FOXO3* SNPs and $PM_{2.5}$ on the repeatedly measured MMSE score. The negative association between $PM_{2.5}$ 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 $PM_{2.5}$ exposure (interaction term for homozygous minor alleles and $PM_{2.5}$ were 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 $PM_{2.5}$ exposure, not under high $PM_{2.5}$ exposure. Participants under the high $PM_{2.5}$ exposure had lower MMSE scores than those under the low $PM_{2.5}$ exposure, and the difference of the predicted MMSE score between low and high $PM_{2.5}$ 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 $PM_{2.5}$ exposure ($PM_{2.5}$ ≤50 μg/m³), but disappeared under high $PM_{2.5}$ exposure (Table 4). The effect of $PM_{2.5}$ 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 $PM_{2.5}$ 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 $PM_{2.5}$ exposure. Carriers of homozygous minor alleles of *FOXO3* SNPs were protected against cognitive decline. Simultaneously, we documented a harmful association between $PM_{2.5}$ exposure and cognitive function. Moreover, we identified a significant gene–environment interaction between *FOXO3* and $PM_{2.5}$ 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 $PM_{2.5}$ 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 $PM_{2.5}$ (0.47 points for *rs2802292* vs −0.20 points for each 10 μg/m³ increase of $PM_{2.5}$). 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 $PM_{2.5}$ 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, $PM_{2.5}$, and MMSE Score at Baseline (Cross-sectional Analyses)

	Model With $PM_{2.5}$			Model With FOXO		Model With $PM_{2.5}$ and FOXO		Model With $PM_{2.5}$, FOXO, and Interaction Term	
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)			4 900						
TC			3 625	0.13 (−0.16, 0.42)	.389	0.07 (−0.22, 0.36)	.648	−0.39 (0.60)	.51
TT			577	0.51 (−0.08, 1.09)	.090	0.38 (−0.20, 0.96)	.202	0.83 (1.14)	.47
$PM_{2.5}$ in 10 μg/m³	−0.39 (−0.5, −0.28)	<.001				−0.38 (−0.49, −0.27)	<.001	−0.41 (0.07)	<.001
rs4946936 TC × $PM_{2.5}$ in 10 μg/m³								0.09 (0.11)	.424
rs4946936 TT × $PM_{2.5}$ in 10 μg/m³								−0.09 (0.23)	.676
rs2802292 (TT as reference)			4 574						
TG			3 777	0.17 (−0.12, 0.46)	.256	0.14 (−0.15, 0.43)	.358	−1.01 (0.60)	.093
GG			751	0.65 (0.12, 1.17)	.015	0.53 (0.01, 1.05)	.048	1.48 (1.02)	.145
$PM_{2.5}$ in 10 μg/m³	−0.39 (−0.5, −0.28)	<.001				−0.38 (−0.49, −0.27)	<.001	−0.45 (0.08)	<.001
rs2802292 TG × $PM_{2.5}$ in 10 μg/m³								0.22 (0.11)	.048
rs2802292 GG × $PM_{2.5}$ in 10 μg/m³								−0.20 (0.20)	.312
rs2253310 (GG as reference)			4 609						
GC			3 747	0.15 (−0.14, 0.45)	.298	0.12 (−0.17, 0.41)	.407	−0.82 (0.60)	.17
CC			746	0.59 (0.07, 1.11)	.027	0.47 (−0.05, 1.00)	.076	1.59 (1.02)	.119
$PM_{2.5}$ in 10 μg/m³	−0.39 (−0.5, −0.28)	<.001				−0.38 (−0.49, −0.27)	<.001	−0.44 (0.08)	<.001
rs2253310 GC × $PM_{2.5}$ in 10 μg/m³								0.19 (0.11)	.103
rs2253310 CC × $PM_{2.5}$ in 10 μg/m³								−0.23 (0.20)	.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.

Table 3. Association of *FOXO3* SNPs, $PM_{2.5}$ and MMSE Score (Longitudinal Analysis)

	Model—$PM_{2.5}$		Model—FOXO		Model—$PM_{2.5}$ + FOXO		Model—$PM_{2.5}$ × FOXO	
Term	Beta (95% CI)	*p*	Beta (95% CI)	*p*	Beta (95% CI)	*p*	Beta (*SE*)	*p*
rs4946936 (CC as reference)								
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
$PM_{2.5}$ 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 × $PM_{2.5}$ in 10 μg/m³							0 (0.08)	.993
rs4946936 TT × $PM_{2.5}$ 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
$PM_{2.5}$ 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 × $PM_{2.5}$ in 10 μg/m³							0.09 (0.08)	.271
rs2802292 GG × $PM_{2.5}$ 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
$PM_{2.5}$ 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 × $PM_{2.5}$ in 10 μg/m³							0.07 (0.08)	.405
rs2253310 CC × $PM_{2.5}$ 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.

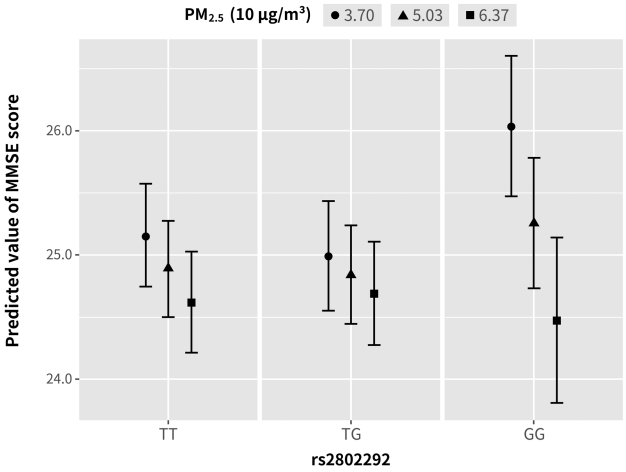

Figure 1. The predicted MMSE score (95% CI) of different genotypes of *rs2802292* at different $PM_{2.5}$ levels. *Notes:* The predicted MMSE score was calculated based on the generalized estimating equation with the interaction term of *rs2802292* and $PM_{2.5}$, adjusting for age, sex, education, residence, marriage, exercise, smoking, and drinking alcohol. The $PM_{2.5}$ level of 3.70, 5.03, and 6.37 were the mean − *SD*, mean, and mean + *SD* of $PM_{2.5}$ (10 μg/m³). MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter.

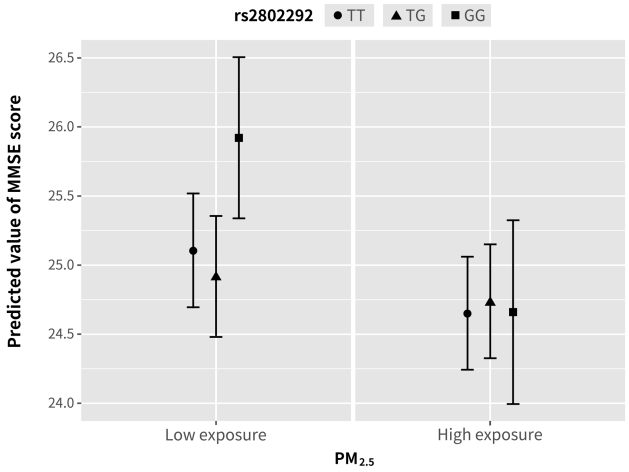

Figure 2. The predicted MMSE score (95% CI) of different $PM_{2.5}$ exposure levels for different genotypes of *rs2802292*. *Notes:* The predicted MMSE score was calculated based on the generalized estimating equation with the interaction term of *rs2802292* and $PM_{2.5}$ group, adjusting for age, sex, education, residence, marriage, exercise, smoking, and drinking alcohol. The cutoff point for the low and high exposure of $PM_{2.5}$ was 50 μg/m³. MMSE = Mini-Mental State Examination; CI = confidence interval; PM = particulate matter.

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 $PM_{2.5}$ 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 $PM_{2.5}$ 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

Table 4. Association Between *FOXO3* and MMSE Score Stratified by High and Low $PM_{2.5}$ Concentration (Longitudinal Analysis)

	$PM_{2.5}$ ≤50 μg/m³			$PM_{2.5}$ >50 μg/m³		
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 (−0.53, 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	0.65 (0.05, 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/m³	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.

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.

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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