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Air pollution exposure during pregnancy and childhood, *APOE* ε4 status and Alzheimer polygenic risk score, and brain structural morphology in preadolescents

Esmée Essers [a,b,c,d], Anne-Claire Binter [a,b,c], Alexander Neumann [d,e,f], Tonya White [d,g], Silvia Alemany [h,i,j], Mònica Guxens [a,b,d,*]

[a] ISGlobal, Barcelona, Spain
[b] Universitat Pompeu Fabra, Barcelona, Spain
[c] Spanish Consortium for Research on Epidemiology and Public Health (CIBERESP), Instituto de Salud Carlos III, Madrid, Spain
[d] Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Centre, Rotterdam, the Netherlands
[e] Complex Genetics of Alzheimer's Disease Group, VIB Center for Molecular Neurology, VIB, Antwerp, Belgium
[f] Department of Biomedical Sciences, University of Antwerp, Antwerp, Belgium
[g] Department of Radiology and Nuclear Medicine, Erasmus Medical Centre, Rotterdam, the Netherlands
[h] Psychiatric Genetics Unit, Group of Psychiatry, Mental Health, and Addiction, Vall d'Hebron Research Institute, Barcelona, Spain
[i] Biomedical Network Research Centre on Mental Health (CIBERSAM), Barcelona, Spain
[j] Department of Psychiatry and Forensic Medicine, Universitat Autònoma de Barcelona, Barcelona, Spain

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ABSTRACT

Background: Air pollution exposure is associated with impaired neurodevelopment, altered structural brain morphology in children, and neurodegenerative disorders. Differential susceptibility to air pollution may be influenced by genetic features.

Objectives: To evaluate whether the *apolipoprotein E (APOE)* genotype or the polygenic risk score (PRS) for Alzheimer's Disease (AD) modify the association between air pollution exposure during pregnancy and childhood and structural brain morphology in preadolescents.

Methods: We included 1186 children from the Generation R Study. Concentrations of fourteen air pollutants were calculated at participants' home addresses during pregnancy and childhood using land-use-regression models. Structural brain images were collected at age 9–12 years to assess cortical and subcortical brain volumes. *APOE* status and PRS for AD were examined as genetic modifiers. Linear regression models were used to conduct single-pollutant and multi-pollutant (using the Deletion/Substitution/Addition algorithm) analyses with a two-way interaction between air pollution and each genetic modifier.

Results: Higher pregnancy coarse particulate matter (PM_{coarse}) and childhood polycyclic aromatic hydrocarbons exposure was differentially associated with larger cerebral white matter volume in *APOE* ε4 carriers compared to non-carriers (29,485 mm^3 (95% CI 6,189; 52,781) and 18,663 mm^3 (469; 36,856), respectively). Higher pregnancy PM_{coarse} exposure was differentially associated with larger cortical grey matter volume in children with higher compared to lower PRS for AD (19436 mm^3 (825, 38,046)).

Discussion: APOE status and PRS for AD possibly modify the association between air pollution exposure and brain structural morphology in preadolescents. Higher air pollution exposure is associated with larger cortical volumes in *APOE* ε4 carriers and children with a high PRS for AD. This is in line with typical brain development, suggesting an antagonistic pleiotropic effect of these genetic features (i.e., protective effect in early-life, but neurodegenerative effect in adulthood). However, we cannot discard chance findings. Future studies should evaluate trajectorial brain development using a longitudinal design.

* Corresponding author. Barcelona Institute for Global Health (ISGlobal) – Campus Mar, Carrer Dr. Aiguader 88. 08003, Barcelona, Spain.
E-mail addresses: esmee.essers@isglobal.org (E. Essers), anneclaire.binter@isglobal.org (A.-C. Binter), alexander.neumann@uantwerpen.vib.be (A. Neumann), t.white@erasmusmc.nl (T. White), silvia.alemany@vhir.org (S. Alemany), monica.guxens@isglobal.org (M. Guxens).

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1. Introduction

Air pollution is one of the biggest environmental and human health threats in the world (Cohen et al., 2017; European Environment Agency, 2020). Studies have assessed the relationship between air pollution exposure and brain structural morphology in children, finding alterations in (sub)cortical brain regions at different ages (Beckwith et al., 2020; Peterson et al., 2015; Guxens et al., 2018; Mortamais et al., 2017; Lubczyńska et al., 2021). Results showed alterations in cortical and subcortical brain regions in relation to exposure to different air pollutants from various sources of traffic-related air pollution (Beckwith et al., 2020; Peterson et al., 2015; Guxens et al., 2018; Mortamais et al., 2017; Lubczyńska et al., 2021). Neuroinflammation and oxidative stress have been shown as potential biological mechanisms behind these associations (Block et al., 2012; Saenen et al., 2019). However, the exact mechanisms by which air pollution exposure is associated with brain development in children are not yet fully understood. Moreover, the interactions between genetics, air pollution, and neurodevelopment have been poorly studied. It is important to investigate genetic interactions in this association as early as childhood, to better understand neurological diseases later in life such as Alzheimer's disease (AD) (Calderón-Garcidueñas et al., 2020). Two possible genetic modifiers are the *apolipoprotein E (APOE)* gene and the polygenic risk score (PRS) for AD.

The *APOE* gene is involved in lipid homeostasis and cholesterol metabolism, and carriers of the epsilon 4 (ε4) allele are at higher risk of neurodegenerative processes (Calderón-Garcidueas et al., 2012; Kloske and Wilcock, 2020; Piers, 2018; Fernandez et al., 2019; Liu et al., 2013). The ε4 allele is the strongest known common genetic risk factor for AD and has been studied as a possible genetic modifier of the association between air pollution and brain morphology (Calderón-Garcidueas et al., 2012; Liu et al., 2013; Alemany et al., 2018; Oudin et al., 2019; Schikowski et al., 2015; Cacciottolo et al., 2017). Animal models identified that accumulation of Aβ amyloid (hallmark of AD) may explain the more prominent association between air pollution exposure and neurodegeneration in carriers of the *APOE* ε4 allele and a recent study found associations between air pollution and AD biomarkers (Cacciottolo et al., 2017; Youmans et al., 2012; Alemany et al., 2021). Most studies have been conducted in adults, finding associations between air pollution exposure and neurodegenerative features in *APOE* ε4 carriers or finding no modifying effect of *APOE* status (Calderón-Garcidueas et al., 2012; Oudin et al., 2019; Schikowski et al., 2015; Cacciottolo et al., 2017; Alemany et al., 2021). Research is limited in children. To the best of our knowledge, only one study evaluated *APOE* ε4 status as a genetic modifier of the association between air pollution exposure during childhood and brain morphology outcomes (Alemany et al., 2018). Authors concluded that higher exposure to polycyclic aromatic hydrocarbons (PAHs) and nitrogen dioxide (NO_2) was more strongly associated with smaller caudate volumes in *APOE* ε4 carriers as compared to non-carriers in Spanish school-aged children (Alemany et al., 2018).

Genome-wide association studies (GWAS) have identified a multitude of genetic loci (single nucleotide polymorphisms, SNP) for many neurodegenerative diseases, including AD. (Bellenguez et al., 2020; Wightman et al., 2021) The PRS for AD is the combination of the disease burden per locus transformed into a single score and is used to assess the genetic burden of an individual to AD. It captures the majority of common SNP effects and thus explains a larger fraction of the SNP heritability as compared to the *APOE* genotype (Lamballais et al., 2020; Leonenko et al., 2021). Few studies have assessed PRS for AD in relation to brain morphology, with only two looking at children and none at air pollution exposure (Lamballais et al., 2020; Lupton et al., 2016; Axelrud et al., 2018; Mormino et al., 2016; Foley et al., 2017).

Since foetuses and children do not have fully developed defence mechanisms, air pollutants could cross the placental- and blood-brain-barrier, alter healthy brain development, and manifest in neurological disorders (Block et al., 2012; Grandjean and Landrigan, 2014; Costa et al., 2017; Bové et al., 2019). Further, early-life processes like brain development could be influenced by the genetic burden for AD, although findings from epidemiological research are inconsistent (Lamballais et al., 2020; Axelrud et al., 2018; Vinueza-Veloz et al., 2020; Shaw et al., 2007; Chang et al., 2016; Quiroz et al., 2015). Some studies have found associations between AD genetic burden and various brain regions in children (Axelrud et al., 2018; Shaw et al., 2007; Chang et al., 2016; Quiroz et al., 2015), but another one conducted in the same cohort as the present study did not (Lamballais et al., 2020). In this study, we aim to evaluate how *APOE* status and PRS for AD modify the association between air pollution exposure during pregnancy and childhood and brain structural morphology in preadolescents. We attempt to replicate the findings of Alemany et al. 2018 and extend them by including more air pollutants, global and subcortical brain volumes, and evaluating the PRS for AD as a potential genetic modifier in a larger sample size.

2. Methods

2.1. Study population and design

The current study is embedded in a population-based birth cohort study based in Rotterdam, the Netherlands: the Generation R Study (Kooijman et al., 2016). The study followed women from pregnancy onward and enrolled a total of 9778 women during pregnancy or right after delivery. Children were born between April 2002 and January 2006. In our study, we included mothers with a singleton pregnancy whose children had available genotype data and a European ethnic origin (N = 2796). Using a European population improves the performance of the PRS and allows for correct assessment of the *APOE* genotype with neurodevelopmental burden, since this is dependent on ancestry (Baker and Escott-Price, 2020; Naslavsky et al., 2022). When children were between 9 and 12 years old, those still involved in the study were invited to participate in a magnetic resonance imaging (MRI) scanning session (White et al., 2018). Among our study population, air pollution data and good quality T_1-weighted imaging data was available for 1186 children (Fig. A1). Ethical approval for the study was granted by the Medical Ethics Committee of the Erasmus Medical Centre in Rotterdam, the Netherlands. Informed consent was given by mothers.

2.2. Air pollution exposure

Concentrations of various air pollutants were estimated for the pregnancy (i.e., from conception until birth) and childhood (i.e., from birth until MRI scanning session) periods using standardized procedures that are detailed in previous literature (Guxens et al., 2018, 2021; De Hoogh et al., 2013; Jedynska et al., 2014; Yang et al., 2015; Beelen et al., 2013). Briefly, two-week measurements were done thrice (warm, cold, and intermediate seasons) spread across the Netherlands and Belgium. NO_2 and nitrogen oxides (NO_x) were measured in 80 sites and particulate matter (PM) with aerodynamic diameter <10 μm (PM_{10}) and <2.5 μm ($PM_{2.5}$) in 40 of these sites. PM with aerodynamic diameter between 10 and 2.5 μm was calculated by subtracting $PM_{2.5}$ from PM_{10} (PM_{coarse}). Absorbance of $PM_{2.5}$ fraction ($PM_{2.5}$ absorbance), oxidative potential of $PM_{2.5}$ (OP) evaluated using two acellular methods: dithiothreitol (OP_{DTT}) and electron spin resonance (OP_{ESR}), and composition of $PM_{2.5}$ consisting of organic carbon (OC), PAHs, copper (Cu), iron (Fe), silicon (Si), and zinc (Zn), were measured from $PM_{2.5}$ filters.

Results of the three two-week measurements were averaged for every pollutant. Those concentrations were adjusted for temporal variability using data from a continuous reference site, resulting in one mean annual concentration for the year of measurement. Then, the concentrations of each air pollutant were estimated at each geocoded address that the study participants have lived at using land use regression (LUR) models. LUR models were built using linear regression models to determine the combination of land use predictors (e.g., traffic intensity on the nearest road, population density) that best explained the annual

concentration level of each pollutant. Considering the time that each participant spent at every address and weighting the pollution concentrations accordingly, the mean air pollution concentration during pregnancy and childhood of each pollutant and for each participant was calculated. For those participants recruited after birth, the address at birth was considered representative for the pregnancy period. Lastly, as historical data of the pollutants was unavailable, extrapolation to the exact periods of study was not possible, and thus the assumption that the spatial contrast remained constant over time was made based on previous studies (Eeftens et al., 2011; Gulliver et al., 2013).

2.3. Brain structural morphology

Prior to the actual MRI scanning session, each child underwent a mock session to familiarize themselves with the environment (White et al., 2018). The MRI scans were performed with a 3 T General Electric scanner (Discovery MR750W, GE Worldwide, Milwaukee, WI) using an 8-channel receive-only head coil. Average age of the child at MRI session was 10.2 years.

Structural T_1-weighted images of the whole brain were collected using the following sequence parameters: TR = 8.77 ms, TE = 3.4 ms, TI = 600 ms, flip angle = 10°, field of view = 220 mm × 220 mm, acquisition matrix = 220 × 220, slice thickness = 1 mm, number of slices = 230, voxel size = 1 mm × 1 mm x 1 mm, and ARC acceleration = 2 (White et al., 2013, 2018). FreeSurfer Image Analysis Suite 6.0 was used to process the MRI data (by conducting cortical reconstruction and volumetric segmentation) and pre-processing steps were taken (Fischl, 2012; Muetzel et al., 2018, 2019). Global brain volumes (cerebral white matter, cortical and subcortical grey matter, corpus callosum, and cerebellum) were extracted and subcortical brain volumes (pallidum, putamen, caudate nucleus, thalamus, amygdala, hippocampus, and nucleus accumbens) were automatically labelled. Volumes across the left and right hemispheres were summed in case of bilateral volumes. To check the processing of the MRI data, visual inspection of FreeSurfer reconstructions was done using a standard protocol (Hibar et al., 2015). Briefly, several slices in all three planes were examined for accuracy of the pial and white matter surfaces. In cases where major problems in the surface reconstructions were observed, data was flagged as unusable and excluded from analyses.

2.4. Genotyping, APOE status, and polygenic risk score

Collection of DNA samples, calling procedures for genotyping, and quality control in the Generation R Study are described elsewhere (Lamballais et al., 2020; Medina-Gomez et al., 2015). To summarize, DNA samples of the child were collected from umbilical cord blood at birth or by venepuncture during a research centre visit when the child was around six years (Medina-Gomez et al., 2015). Genotyping was done using Illumina HumanHap 610 or 660 Quad chips and PLINK was used for DNA quality control (Medina-Gomez et al., 2015). Data has been imputed to the HRC 1.1 reference with the Michigan Imputation Service (Das et al., 2016). SNPs with a minor allele frequency below 1% or imputation quality (R^2) below 0.80 were excluded from further analysis.

The *APOE* status of each participant was determined based on the allelic combinations of two SNPs: rs429358 and rs7412. These SNPs represent the major *APOE* allelic variants ε2, ε3, and ε4. The ε4 allele is defined by presence of the cytosine allele at both SNPs (Radmanesh et al., 2014). Children were classified as an *APOE* ε4 carrier if they had at least one cytosine allele at both SNP locations and as a non-carrier if they had two thymine alleles in at least one SNP location (Table A1).

The polygenic scores were based on a large GWAS for AD (Jansen et al., 2019). PRSice-2 was used to compute the PRS (Choi and O'Reilly, 2019). This method sums up the number of SNP alleles that are carried by the participant, weighted by the SNP allele effect size estimated in previous GWAS. We used a clumping procedure to only consider independent SNPs using the default PRSice settings of a 0.1 r^2 threshold within a 250 kb window. The *APOE* region was excluded (chr19: 44.4–46.5 Mb), as previous research has shown that prediction accuracy is improved when *APOE* is modelled separately as opposed to being included in the PRS (Leonenko et al., 2021). To avoid including SNPs in the PRS with uncertain associations with AD, we only included 233 independent SNPs (Table A2), which previously showed robust evidence for an association with AD ($p < 0.000035$). This threshold was chosen, as it was the most optimal for predicting AD in the original GWAS (Jansen et al., 2019). The PRS were standardized to a mean of 0 and a standard deviation (SD) of 1.

2.5. Potential confounding variables

The potential confounding variables were defined a priori based on previous literature and the availability of information on the variables in the Generation R Study (Guxens et al., 2018, 2021; Lubczyńska et al., 2020, 2021; Alemany et al., 2018). Parental age at enrolment (years), psychological distress during pregnancy (measured using the Brief Symptom Inventory (Derogatis, 1993)), education level during pregnancy (primary, secondary, or higher), and country of birth (The Netherlands or other), maternal smoking during pregnancy (never, until pregnancy known, or continued use during pregnancy), alcohol use during pregnancy (never, until pregnancy known, or continued use during pregnancy), and parity (nulliparous, one child, or two or more children), monthly household income (<900€, 900–1600€, 1600–2200€, or >2200€) and family status (parents married, parents living together, or mono-parental) were collected by questionnaires during pregnancy. Parental weight and height (in kg and cm, respectively) were measured or self-reported in the first trimester of the pregnancy, and body mass index (BMI) was subsequently calculated (kg/cm^2). Child sex (boy or girl) was collected from hospital records. Maternal intelligence quotient (IQ) score was assessed at child's age of six years using the Ravens Advanced Progressive Matrices test, set I (Raven, 1962). Child age (years) was collected at the MRI scanning session.

2.6. Statistical analysis

Missing values of potential confounding variables were imputed 25 times to limit selection bias following standard procedures for multiple imputation (Table A3). (Sterne et al., 2009; Spratt et al., 2010) This was done on the dataset including subjects that have data available on exposure, outcome, and genetic information (N = 1186). The percentage of missing values was below 19% for all potential confounding variables. Distributions in the imputed and observed datasets were comparable (Table A4).

Children included in the analyses were more likely to be younger, have parents that were older and had a higher education, have mothers who had a lower psychological distress, never smoked during pregnancy, and have a higher IQ, and a family with a higher monthly household income than those not included (Table A5). To correct for selection bias, inverse probability weighting was performed by using information available for the European ancestry population (N = 2796) to predict the probability of participation in the present study. The inverse of those probabilities were used as weights in analyses to ensure representative results for the initial European population (Weisskopf et al., 2015; Weuve et al., 2012). The variables used to create the weights and the distribution of the weights can be found in Table A6 and Fig. A2.

We analysed the potential modifying effect of *APOE* ε4 status or PRS for AD on the association between air pollution exposure and brain morphology outcomes following a two-step approach. In the first step, we ran single-pollutant models of each air pollutant during pregnancy and childhood separately with each global and subcortical brain volume, using multiple linear regression models that include a two-way interaction between *APOE* ε4 status or PRS for AD and each air pollutant. In the second step, we ran a multi-pollutant analysis including

all 14 pollutants during pregnancy and childhood separately and the two-way interaction term between the genetic modifier and each air pollutant using the Deletion/Substitution/Addition (DSA) algorithm on the twentieth imputed dataset (Methods A1) (Agier et al., 2016). We only performed the multi-pollutant analysis for a specific outcome if an interaction term was found statistically significant ($p < 0.05$) for at least one pollutant associated with that outcome in the single-pollutant models. The DSA algorithm is an iterative selection method that selects the variables most predictive of the outcome by cross-validation, considering the correlation matrix, and correcting for multiple testing. In case of the correlation between two pollutants being >0.90, only the pollutant with the better LUR model performance (higher R^2 value) was included to avoid collinearity. Following this criterion, PM_{10}, Fe, and OP_{ESR} were excluded for pregnancy and PM_{10}, Fe, and NO_2 for childhood. Since the DSA algorithm is based on cross-validation, each DSA model was run 200 times and the final models were selected based on a frequency of occurrence of 10% of the time or more.

All models were first unadjusted, and then adjusted for all potential confounding variables described above. The assumptions of the linear regression models were fulfilled, which included linearity between each exposure and each outcome, as well as the normality of the residuals, homoscedasticity, non-collinearity of covariates, and the absence of influential observations. Models for subcortical grey matter, corpus callosum, cerebellum, and the other subcortical brain volumes were additionally adjusted for intracranial volume to ensure relativity to head size. This was not done for cerebral white matter and cortical grey matter volumes due to the high correlations with intracranial volume (>0.80). If we observed an association between an air pollutant and cerebral white matter or cortical grey matter volume after the multi-pollutant analysis, we re-ran the association between that air pollutant and intracranial volume to explore if the observed association with white matter or cortical grey matter volume could be explained by a global effect. As a sensitivity analysis, we performed a linear regression model that included all pregnancy and childhood exposures that were significant predictors of a specific outcome in the multi-pollutant analysis. Statistical analyses were carried out using R (version 3.6.0, R Core Team (2019)).

3. Results

Mothers were 32.2 years on average, mostly had a high education (69.3%), and were Dutch (88.0%), and the monthly income was high for 81.1% of households (Table 1). *APOE* ε4 carriers had mothers with a higher IQ score and children with a higher PRS for AD had mothers with a higher IQ score, who had more children, and a family with a higher monthly household income (Table A7). A total of 28.5% were *APOE* ε4 carriers (Table 1 and A1). Median levels of exposure for $PM_{2.5}$ were 17.0 and 16.7 μg/m^3 and for NO_2 were 34.4 and 31.6 μg/m^3 during pregnancy and childhood, respectively (Table 2). Correlations between the air pollutants during pregnancy and childhood were moderate and range from 0.43 for NO_2 to 0.62 for PAHs (Table 2). Correlations among the air pollutants ranged from 0.03 (between PAHs and OP_{ESR} during childhood) to 0.98 (between $PM_{2.5}$ absorbance and PM_{10} during both pregnancy and childhood) (Fig. A3).

3.1. APOE as a genetic modifier

In the single-pollutant analysis, higher exposure to several air pollutants during pregnancy was differentially associated with larger cerebral white matter, cortical grey matter, and corpus callosum volumes, and with smaller subcortical grey matter, putamen, thalamus, hippocampus, and nucleus accumbens volumes in *APOE* ε4 carriers compared to non-carriers (interaction *P*-value < 0.05, Table 3 and A8). Results in Table 3 only show the significant associations found in the single-pollutant analyses, exhaustive associations for all other exposures and outcomes can be seen in Table A8. Following the multi-pollutant analysis, only PM_{coarse} exposure during pregnancy remained differentially associated with larger cerebral white matter volume in *APOE* ε4 carriers compared to non-carriers (29,485 mm^3 (95% CI 6,189; 52,781) per 5 μg/m^3 increase in PM_{coarse}). Cerebral white matter volume was 958 mm^3 (95% CI -14,264; 12,348) smaller in non-carriers and 28,527 mm^3 (95% CI 9,125; 47,928) larger in *APOE* ε4 carriers per 5 μg/m^3 increase in PM_{coarse} (Fig. 1, Table 3 and A9).

Regarding air pollution exposure during childhood, in the single-pollutant analysis, higher exposure to several air pollutants was differentially associated with larger cerebral white matter and cortical grey matter volumes, and with smaller subcortical grey matter, cerebellum, putamen, caudate nucleus, thalamus, and nucleus accumbens volumes in *APOE* ε4 carriers compared to non-carriers (Table 3 and A8). Results in Table 3 only show the significant associations found in the single-pollutant analyses, exhaustive associations for all other exposures and outcomes can be seen in Table A8. Following the multi-pollutant

Table 1
Characteristics of the study population (N = 1186).

GeneticFeatures	
APOE ε4 carriers	28.5
PRS for AD[a]	2.1 ± 6.0
Maternal Characteristics	
Age (years)	32.2 ± 4.0
Pre-pregnancy BMI (kg/m^2)	23.0 ± 3.8
Psychological distress	0.2 ± 0.2
Education level	
Primary education or lower	0.9
Secondary education	29.8
Higher education	69.3
Country of birth	
The Netherlands	88.0
Other	12.0
Smoking during pregnancy	
Never	79.2
Until pregnancy known	10.0
Continued use	10.8
Alcohol during pregnancy	
Never	27.9
Until pregnancy known	15.7
Continued use	56.4
Parity	
0 child	60.7
1 child	31.4
2+ children	7.9
IQ score	102.1 ± 12.1
PartnerCharacteristics	
Age (years)	34.1 ± 4.7
Pre-pregnancy BMI (kg/m^2)	25.0 ± 3.2
Psychological distress	0.1 ± 0.1
Education level	
Primary education or lower	2.3
Secondary education	34.3
Higher education	63.4
Country of birth	
The Netherlands	89.5
Other	10.5
HouseholdCharacteristics	
Monthly household income (€)	
<900	0.8
900 - 1600	5.6
1600 - 2200	12.5
>2200	81.1
Family status	
Parents married	50.9
Parents living together	45.2
Mono parental	3.9

[a] value in 10^{-4}. Abbreviations: *APOE*, *Apolipoprotein E*, BMI, body mass index, IQ, intelligence quotient, PRS for AD, polygenic risk score for Alzheimer's disease. Values are percentage for categorical and mean ± standard deviation for continuous variables.

Table 2
Levels of air pollution exposure during pregnancy and childhood in the study population (N = 1186).

Pollutant	Units	Pregnancy			Childhood			Correlation[a]
		median	p25	p75	median	p25	p75	
NO_2	($\mu g/m^3$)	34.4	32.0	37.2	31.6	28.5	34.8	0.43
NO_X	($\mu g/m^3$)	48.4	40.0	62.6	42.4	37.5	51.9	0.53
PM_{10}	($\mu g/m^3$)	26.9	26.1	28.4	26.2	25.5	27.3	0.50
$PM_{2.5}$	($\mu g/m^3$)	17.0	16.6	17.3	16.7	16.5	17.1	0.55
PM_{coarse}	($\mu g/m^3$)	10.0	9.1	10.7	9.2	8.4	10.1	0.50
$PM_{2.5}$ abs	($10^{-5}m^{-1}$)	1.6	1.5	1.8	1.5	1.4	1.7	0.50
OP_{DTT}	(nmol DTT/min/m^3)	1.3	1.2	1.4	1.3	1.2	1.3	0.54
OP_{ESR}	(arbitrary units/m^3)	1029.1	995.7	1107.4	1002.0	947.4	1061.8	0.55
OC	($\mu g/m^3$)	1.8	1.5	2.0	1.6	1.3	1.8	0.55
PAHs	(ng/m^3)	0.9	0.7	1.1	0.9	0.8	1.1	0.62
Cu	(ng/m^3)	4.7	4.4	5.0	4.5	4.1	4.9	0.50
Fe	(ng/m^3)	119.7	113.8	130.6	114.4	102.9	125.2	0.49
Si	(ng/m^3)	88.6	87.8	90.7	88.4	87.5	90.4	0.56
Zn	(ng/m^3)	19.2	17.7	21.9	18.7	17.3	21.3	0.54

Abbreviations: Cu, copper; Fe, iron; NO_2, nitrogen dioxide; NO_x, nitrogen oxides; OC, organic carbon; OP, oxidative potential (evaluated using two acellular methods: OP_{DTT}; dithiothreitol and OP_{ESR}, electron spin resonance); p25, 25th percentile; p75, 75th percentile; PAHs, polycyclic aromatic hydrocarbons; PM, particulate matter with different aerodynamic diameters: <10 μm (PM_{10}); <2.5 μm ($PM_{2.5}$), and between 10 μm and 2.5 μm (PM_{coarse}); $PM_{2.5\ abs}$, absorbance of $PM_{2.5}$ filters; Si; silicon, Zn; zinc.

[a] Pearson's correlation between the air pollution exposure during the pregnancy and childhood periods.

analysis, only PAHs exposure during childhood remained differentially associated with larger cerebral white matter volume in *APOE* ε4 carriers compared to non-carriers (18,663 mm^3 (95% CI 469; 36,856) per 5 $\mu g/m^3$ increase in PAHs). Cerebral white matter volume was 826 mm^3 (95% CI −8,733; 10,385) larger in non-carriers and 19,488 mm^3 (95% CI 3,894; 35,083) larger in *APOE* ε4 carriers per 5 $\mu g/m^3$ increase in PAHs (Fig. 1, Table 3 and A9).

No associations were found between pregnancy PM_{coarse} or childhood PAHs and intracranial volume (Table A10). When we analysed simultaneously PM_{coarse} exposure during pregnancy and PAHs exposure during childhood with cerebral white matter, we found that PM_{coarse} remained associated with larger cerebral white matter volume (25,125 mm^3 (95% CI 937; 49,313) per 5 $\mu g/m^3$ increase in PM_{coarse}). Childhood exposure to PAHs was no longer associated with larger cerebral white matter volume (13,259 mm^3 (95% CI −5,610; 32,129) per 5 $\mu g/m^3$ increase in PAHs).

3.2. PRS for AD as a genetic modifier

In the single-pollutant analysis, higher exposure to several air pollutants during pregnancy was differentially associated with larger cortical grey matter volume and smaller subcortical grey matter, pallidum, putamen, thalamus, amygdala, and hippocampus volumes for each increase in PRS for AD (Table 3 and A8). Results in Table 3 only show the significant associations found in the single-pollutant analyses, exhaustive associations for all other exposures and outcomes can be seen in Table A8. Following the multi-pollutant analysis, only PM_{coarse} remained differentially associated with larger cortical grey matter volume per increment increase in PRS for AD (19,436 mm^3 (95% CI 825; 38,046) per 5 $\mu g/m^3$ increase in PM_{coarse}). Cortical grey matter volume was 739 mm^3 (95% CI -12,070; 13,548) larger in children with an average PRS for AD and 12,380 mm^3 (95% CI -1,091; 25,852) larger in children with a high PRS for AD per 5 $\mu g/m^3$ increase in PM_{coarse} (Fig. 1, Table 3 and A9).

Higher air pollution exposure during childhood was differentially associated with smaller subcortical grey matter, corpus callosum, cerebellum, pallidum, putamen, caudate nucleus, thalamus, and hippocampus volumes for each increase in PRS for AD in the single-pollutant analysis (Table 3 and A8). No associations remained after the multi-pollutant analysis.

No associations were found between pregnancy PM_{coarse} or childhood PAHs and intracranial volume (Table A10).

4. Discussion

Our study found a possible modifying effect of *APOE* status and the PRS for AD on the association between air pollution exposure during early-life and brain structural morphology in preadolescents. PM_{coarse} exposure during pregnancy and PAHs exposure during childhood were associated with larger cerebral white matter volume in *APOE* ε4 carriers compared to non-carriers. When adjusting simultaneously for PM_{coarse} exposure during pregnancy and PAHs exposure during childhood, only PM_{coarse} remained associated with larger cerebral white matter volume. PM_{coarse} exposure during pregnancy was associated with larger cortical grey matter volume in children with higher PRS for AD.

Our findings on the possible effect modification of the *APOE* genotype and PRS for AD are in the unexpected direction. Previous research in independent cohorts has shown that white and grey matter volumes generally increase throughout infancy and childhood, with most grey matter volumes peaking at adolescence (Lubczyńska et al., 2021; Wierenga et al., 2014, 2018; Lenroot and Giedd, 2006; Tiemeier et al., 2010; Herting et al., 2018). Typical developmental trends for total white matter and cortical grey matter volume in children aged 9–12 years are therefore expected to be increasing. Further, previous research has shown that increases in air pollution exposure is associated with smaller cortical grey and white matter volumes, and we hypothesized that the genetic modifiers amplify this (Beckwith et al., 2020; Peterson et al., 2015). Thus, our findings introduce the possibility of a beneficial effect of the genetic modifiers in early life. This would align with the antagonistic pleiotropic effect hypothesis proposed for the *APOE* gene, which encompasses the idea that genetic modifiers show benefits during early life, but become risk factors for adverse neurodevelopment with increasing age (Mondadori et al., 2007). Alemany et al. 2018 also found that higher air pollution exposure during childhood was associated with smaller caudate nucleus volume in *APOE* ε4 carriers in Spanish children aged 7–11 years. Even if smaller caudate was associated with lower cognitive function and more behavioural problems in their study, the decrease in caudate nucleus volume in this age range is in line with typical development in preadolescence (Alemany et al., 2018; Wierenga et al., 2014). Thus, the findings from Alemany et al. 2018 would also be in line with a potential antagonistic pleiotropic effect (Alemany et al., 2018). Research on the effects of the *APOE* genotype on cognitive function has also highlighted this possible beneficial role of the ε4 allele in early life, although this was not confirmed in a meta-analysis (Mondadori et al., 2007; Ihle et al., 2012). The antagonistic pleiotropic effect hypothesis could explain why we see changes in brain morphology

Table 3
Differential associations between an increment increase in air pollution exposure during pregnancy or childhood with global brain volumes (mm^3) for *APOE* status **(A)** or PRS for AD **(B)** (N = 1186). Only associations with a significant two-way interaction term between *APOE* status **(A)** or PRS for AD **(B)** and air pollution are reported here[a].

	(A) *APOE* status				(B) PRS for AD			
	Pregnancy		*Childhood*		*Pregnancy*		*Childhood*	
	Coef.	*95% CI*	*Coef.*	*95% CI*	*Coef.*	*95% CI*	*Coef.*	*95% CI*
Cerebral WhiteMatter								
NO_X	8,176	(1,494; 14,858)	8,199	(1,071; 15,327)	–	–	–	–
PM_{10}	34,920	(4,006; 65,833)	–	–	–	–	–	–
$PM_{2.5}$	50,168	(8,877; 91,458)	76,629	(22,919; 130,338)	–	–	–	–
PM_{coarse}	**29,485**	**(6,189; 52,781)**[b]	25,940	(1,264; 50,615)	–	–	–	–
$PM_{2.5\ abs}$	–	–	17,987	(246; 35,729)	–	–	–	–
PAHs	–	–	**18,663**	**(469; 36,856)**[b]	–	–	–	–
Cortical Grey Matter								
NO_2	–	–	11,369	(2,387; 20,350)	–	–	–	–
NO_X	9,674	(2,516; 16,831)	9,340	(1,712; 16,968)	–	–	–	–
PM_{10}	39,401	(6,278; 72,524)	40,025	(4,423; 75,627)	–	–	–	–
$PM_{2.5}$	52,202	(7,967; 96,437)	81,646	(24,144; 139,148)	–	–	–	–
PM_{coarse}	28,933	(3,956; 53,910)	–	–	**19,436**	**(825; 38,046)**[b]	–	–
$PM_{2.5\ abs}$	18,015	(338; 35,691)	25,135	(6,168; 44,101)	–	–	–	–
PAHs	–	–	–	–	–	–	–	–
Si	–	–	35,727	(2,367; 69,087)	–	–	–	–
Subcortical Grey Matter								
NO_2	-906	(-1,569; -243)	–	–	-992	(-1,531; -453)	–	–
NO_X	-616	(-1,054; -178)	–	–	-536	(-868; -204)	-423	(-789; -56)
PM_{10}	-2,970	(-4,995; -946)	-2,612	(-4,791; -433)	-2,119	(-3,614; -625)	-1,976	(-3,652; -300)
$PM_{2.5}$	-3,438	(-6,147; -729)	-5,290	(-8,815; -1,766)	-2,501	(-4,424; -579)	-3,945	(-6,585; -1,305)
$PM_{2.5\ abs}$	-1,610	(-2,690; -529)	-1,366	(-2,530; -202)	-1,431	(-2,293; -570	-1,392	(-2,406; -378)
OP_{ESR}	-1,904	(-3,619; -189)	-2,021	(-3,821; -222)	-2,073	(-3,540; -607)	-2,047	(-3,817; -277)
Cu	-2,432	(-4,286; -578)	-2,670	(-4,665; -676)	-2,060	(-3,523; -596)	-1,803	(-3,494; -112)
Fe	-1,986	(-3,536; -435)	-1,774	(-3,189; -359)	-1,732	(-2,927; -538)	–	–
Si	-2,316	(-4,121; -512)	-2,760	(-4,801, -720)	-2,140	(-3,523; -756)	-2,574	(-4,474; -673)
Zn	-1,021	(-1,793; -248)	–	–	-591	(-1,170; -12)	–	–
Corpus Callosum								
NO_X	80	(11, 149)	–	–	–	–	–	–
PM_{10}	390	(70, 710)	–	–	–	–	–	–
PM_{coarse}	319	(79, 559)	–	–	–	–	–	–
Cu	–	–	–	–	–	–	-266	(-533; 0)
Cerebellum								
$PM_{2.5}$	–	–	-12,733	(-25,391; -76)	–	–	-10,462	(-19,930; -994)

Abbreviations: Coef. Coefficient, *APOE, apolipoprotein E*, CI, confidence interval, Cu, copper, Fe, iron, NO_2, nitrogen dioxide, NO_x, nitrogen oxides, OP_{ESR}, oxidative potential evaluated using electron spin resonance, PAHs, polycyclic aromatic hydrocarbons, PM, particulate matter with aerodynamic diameters: less than 10 μm (PM_{10}), less than 2.5 μm ($PM_{2.5}$), and between 10 μm and 2.5 μm (PM_{coarse}), $PM_{2.5\ abs}$, absorbance of $PM_{2.5}$, PRS for AD, polygenic risk score for Alzheimer's disease, Si, silicon, Zn, zinc. Coefficients and 95% CI were obtained with multiple linear regression analyses and calculated per increments of: 10 $\mu g/m^3$for PM_{10}, 5 $\mu g/m^3$for $PM_{2.5}$ and PM_{coarse}, 10^{-5} $m^{-1}$for $PM_{2.5\ abs}$, 10 $\mu g/m^3$for NO_2, 20 $\mu g/m^3$for NO_X, 1000 arbitrary units/$m^3$for OP_{ESR}, 1 $ng/m^3$for PAHs, 5 $ng/m^3$for Cu, 100 $ng/m^3$for Fe, 100 $ng/m^3$for Si, and 10 $ng/m^3$for Zn. Models were adjusted for parental age, psychological distress, education level, and country of birth, maternal smoking and alcohol use during pregnancy, monthly household income, family status, parental height and body mass index, child gender, maternal intelligence quotient, and child age at magnetic resonance imaging session. Models for subcortical grey matter, corpus callosum, and cerebellum were also adjusted for intracranial volume.

[a] Exhaustive results for all pollutants and outcomes can be found in Table A8.

[b] Associations that remained after the multi-pollutant analysis with the Deletion/Substitution/Addition algorithm.

volumes in line with typical development with increased exposure to some air pollutants in *APOE* ε4 carriers and children with higher PRS for AD. However, not all research is in line with this hypothesis, and therefore it should be interpreted with caution (Henson et al., 2020). While some studies have found associations between the genetic burden for AD and various brain regions (Axelrud et al., 2018; Shaw et al., 2007; Chang et al., 2016; Quiroz et al., 2015), a previous study including children from the Generation R Study found no evidence of a link between the *APOE* genotype with global brain structure in children, as well as no evidence of a genetic burden of PRS for AD on cognitive functioning throughout childhood (Lamballais et al., 2020). Research further suggests that the genetic burden of the *APOE* genotype might only be apparent in adulthood or old age as a result of cumulative processes (Lamballais et al., 2020). Therefore, the neurodegenerative impacts of air pollution exposure might only become measurable after early life (Lamballais et al., 2020; Tzioras et al., 2019). Another possible explanation could be that the observed associations reflect accelerated maturation for age and thus eventually in later life an earlier decline. In order to test this hypothesis we would need longitudinal data, preferably over the lifespan, although accelerated longitudinal models could potentially address this question (Kelly et al., 2022). However, the brain volumes of our study sample generally fall in line with the expected range for normal development, and therefore our data do not indicate much larger volumes than expected (Lubczyńska et al., 2021; Wierenga et al., 2014, 2018; Lenroot and Giedd, 2006; Tiemeier et al., 2010; Herting et al., 2018). To further understand the role of these genetic modifiers in neurodevelopment, future studies include older ages and combine neuroimaging and neuropsychological assessments for a more exhaustive brain morphology measurement.

Several air pollutants were associated with brain morphology in the single-pollutant models with a modifying effect of *APOE* status or PRS for AD, but only PM_{coarse} during pregnancy and PAHs during childhood were selected in the multi-pollutant analysis. Further, simultaneous analysis of these two pollutants with cerebral white matter volume

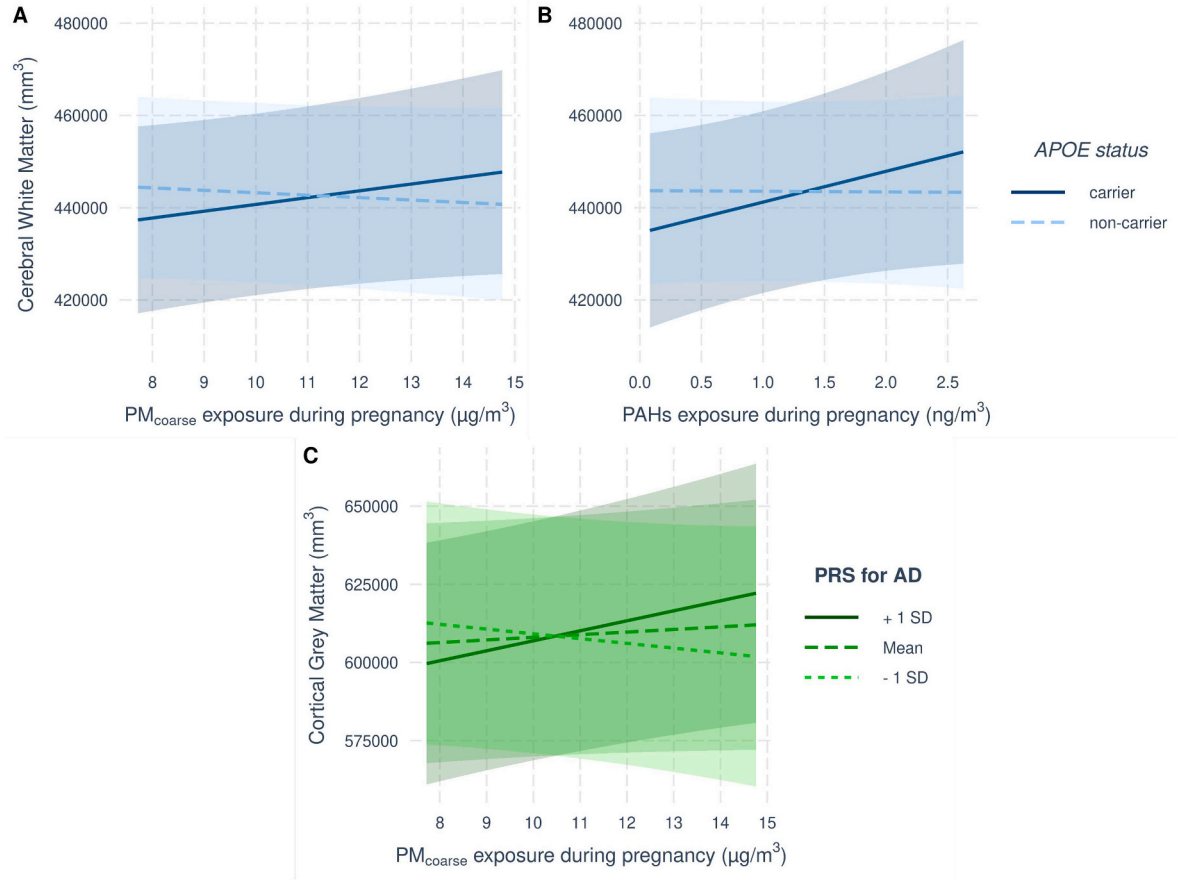

Fig. 1. Associations between PM_{coarse} exposure during pregnancy **(A)** or PAHs exposure during childhood **(B)** and cerebral white matter volume according to *APOE* status, and between PM_{coarse} exposure during pregnancy and cortical grey matter volume according to PRS for AD **(C)** from the twentieth imputed dataset (n = 1186). Only the remaining associations with a significant two-way interaction term between *APOE* status **(A, B)** or PRS for AD **(C)** after the multi-pollutant analysis are shown.

Coefficients and 95% confidence interval were obtained with multiple linear regression analyses. **(A)** and **(B)** show the associations for *APOE* ε4 carriers (solid line) and non-carriers (dashed line) and **(C)** shows the associations for the mean (large, dashed line), +1 SD (solid line), and −1 SD (small, dashed line) PRS for AD. Shaded areas indicate the 95% confidence interval. A positive slope indicates that an increase in the air pollutant is associated with larger structural brain morphology volumes. The intersect is at the air pollution level where the sum of the air pollutant and genetic effect is null. Models were adjusted for parental age, psychological distress, education level, and country of birth, maternal smoking and alcohol use during pregnancy, monthly household income, family status, parental height and body mass index, child gender, maternal intelligence quotient, and child age at magnetic resonance imaging session. Plots are comparable for all other imputed datasets [data not shown]. Abbreviations: *APOE, apolipoprotein E,* PAHs, polycyclic aromatic hydrocarbons, PM, particulate matter with aerodynamic diameter between 10 µm and 2.5 µm (PM_{coarse}), PRS for AD, polygenic risk score for Alzheimer's disease, SD, standard deviation.

showed that the exposure to PM_{coarse} during pregnancy seems to be more relevant. PM_{coarse} is mainly emitted from anthropogenic sources like motor vehicles or fuel combustion (U.S. EPA, 2019). Exposure to PM_{coarse} has shown HPA-axis activation and gene expression alterations in the brain (Block et al., 2012; U.S. EPA, 2019; Suades-González et al., 2015). Exposure to PAHs, which are released as a by-product when there is an incomplete combustion of fossil fuels, has been linked to neurodevelopmental disorders (Guxens et al., 2021; Suades-González et al., 2015). In contrast to current literature that highlights the adverse effects of these pollutants on the brain, our results show that increased exposure of these specific pollutants is related to typical neurodevelopment in *APOE* ε4 carriers and children with a higher PRS for AD. It is important to recognize that exposure to solely PM_{coarse} and PAHs does not reflect actual air quality conditions, since humans are exposed to a wide mixture of primary and secondary air pollutants (U.S. EPA, 2019). In our study, PM_{coarse} and PAHs may be considered more as markers of traffic-related air pollution. A possible explanation as to why these pollutants were selected in the multi-pollutant models could be due to factors related to the statistical approach used as opposed to true biological reasons.

Our study has several important strengths. We included a neuroimaging study with a large sample size providing precise measurements of brain regions. We used advanced statistical methods to limit possible selection and attrition bias through multiple imputation and inverse probability weighting. Many socioeconomic and lifestyle factors that have shown to potentially be associated with air pollution and brain outcomes in children were adjusted for. Exposure levels during two critical periods of development for a large amount of air pollutants were calculated through standardized assessment methods that considered changes of residency. Also, the interaction between air pollution and the genetic modifiers could be assessed simultaneously in an advanced multi-pollutant approach that corrected for multiple testing.

There are also some limitations that merit discussion. In spite of the relatively large sample size, we recognize the possibility of our associations being due to chance because of difficulties in accurately finding and replicating results when looking at interactions between genes and environmental factors (Duncan et al., 2014; Duncan and Keller, 2011). First, our single-pollutant analyses strategy encompassed many models for each genetic modifier. We adjusted for multiple testing related to the exposures using the DSA algorithm, which has shown good performance in false discovery proportion. However, we did not adjust for multiple

testing related to the outcomes and having many testable hypotheses increases the possibility of chance findings (Duncan et al., 2014; Khoury, 2017). When interpreting results we also need to take into account the large number of non-significant associations that were shown. Second, even though the sample size was larger as compared to previous studies, it may have been too small to detect real genetic effects reliably, since detecting interactions effect require large sample sizes (into the ten-to hundred-thousandths) to improve reliability of findings (Munafò et al., 2014). Third, difficulties lie in the rudimentary understanding of genotype-to-phenotype pathways in neurological disorders, especially with regards to the limited knowledge on the modifying effect of genetic features on the association between air pollution exposure and brain morphology. The pathways for gene-and-environment interactions are extremely complex and disorders like AD are likely influenced by a multitude of genetic variants. Thus, even though we included both *APOE* status and the PRS for AD, two of the most prominent common genetic factors related to AD, this is likely not enough to explain the genetic complexity of the disorder. Further, even though 233 SNPs that showed robust evidence for AD were included in the PRS for AD, most SNPs may have limited relevance to brain development which may limit the interactions we see. Also, the *APOE* ε2 allele is known to be neuroprotective, but we were unable to use these subjects in a sensitivity analysis to further understand the possible modifying effect of the *APOE* genotype due to the small sample size (N = 7) (Goldberg et al., 2020). Another limitation is related to the air pollution concentrations which were determined when children were between 3.5 and 9 years of age. We were unable to extrapolate air pollution levels to the specific periods of interest, introducing the possibility of exposure misclassification (especially during the pregnancy period) due to temporal variation. However, the assumption was made that the concentrations of the pollutants remained stable over time based on research supporting this done in the Netherlands between 1999 and 2007 and in Great Britain between 1991 and 2009 (Eeftens et al., 2011; Gulliver et al., 2013). Lastly, our outcome assessment was based on a single measurement from the MRI scanning session. Combining neuroimaging and neuropsychological assessments and including repeated measurements and a longitudinal analysis design throughout childhood, adulthood and old age in future studies can help elucidate how the trajectory of the brain structure development is affected by air pollution exposure and the potential genetic modifiers. This might give a clearer idea of how the associations evolve over time, especially since neurological impacts of air pollution exposure and the burden of genetic modifiers may follow a cumulative trend that only becomes measurable in the course of time.

In conclusion, we found evidence suggesting genetic susceptibility of both *APOE* status and PRS for AD on the association between air pollution exposure and brain structural morphology in preadolescent children from the Generation R Study. Both genetic factors seem to modify the association towards the typical development of the brain, which was unexpected but in line with antagonistic pleiotropic effects. The brain is a complex organ that undergoes many developmental changes, making interpretation of results with regards to healthy versus pathological development difficult (Block et al., 2012). Nevertheless, our study is one of the first to attempt to characterize gene-environment interactions related to air pollution and brain morphology in children. Future studies should research the possibility of the antagonistic pleiotropic effect and consider a longitudinal design for studying the trajectory of brain development.

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Credit author statement

Esmée Essers: conceptualization, formal analysis, methodology, writing – original draft, visualization. **Anne-Claire Binter:** conceptualization, methodology, writing – review & editing, visualization, supervision. **Alexander Neumann:** methodology, writing – review & editing. **Tonya White:** writing – review, & editing, funding acquisition. **Silvia Alemany:** conceptualization, writing – review & editing. **Mònica Guxens:** conceptualization, methodology, writing – review & editing, supervision, funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The data that has been used is confidential.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2022.114595.

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