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The mediating role of the gut microbiome in the association between ambient air pollution and autistic traits

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ABSTRACT

Air pollution has been reported to be an environmental risk factor for autism spectrum disorder. However, the gut microbiome's role as a potential mediator has not been investigated. We aimed to clarify whether particulate matter with an aerodynamic diameter ≤10 μm (PM_{10}) and nitrogen dioxide (NO_2) exposure impact autistic traits through the gut microbiome. Using 170 mother–child pairs, PM_{10} and NO_2 exposure levels during pregnancy (1st, 2nd, and 3rd trimesters) and annual residential PM_{10} levels at age 2, 4, and 6 years were estimated. Autistic traits and gut microbiome were assessed at age 6 years. The associations of PM_{10} or NO_2 exposure, gut microbiome composition, and autistic traits were explored, and mediation analyses of statistically significant findings were also conducted. Exposure to PM_{10} during the 1st trimester of pregnancy was associated with increased autistic traits (10.6% change per interquartile range (IQR) increase, 95% confidence interval [CI]: 1.1, 21.0) and with *Proteobacteria* relative abundance at age 6 years (66.9% change per IQR increase, 95% CI: 21.3, 129.8). First trimester NO_2 exposure was associated with autistic traits (12.1% change, 95% CI: 0.1, 25.5) and *Proteobacteria* relative abundance at age 6 years (48.1% change, 95% CI: −0.1, 119.6). *Proteobacteria* relative abundance was related to autistic traits (4.4% change per 2-fold increase, 95% CI: 1.3, 7.5). Relations between PM_{10} or NO_2 exposure during the 1st trimester and autistic traits at age 6 years were partially mediated by *Proteobacteria* (proportion mediated 23.2%, $p = 0.01$ and 16.7%, $p = 0.06$; respectively). PM_{10} and possibly NO_2 exposure during early pregnancy may affect autistic traits at age 6 years through the alteration of *Proteobacteria* abundance.

1. Introduction

Autism spectrum disorder (ASD) affects one in 44 children in the United States (Maenner et al., 2021), and is marked by deficits in social communication, restricted interests, and repetitive behavior (American Psychiatric Association, 2013). Autistic traits are detectable between 6 and 18 months (Barbaro and Dissanayake, 2009), indicating that critical windows to genetic and environmental factors occur during prenatal and early postnatal periods. Although the high heritability of ASD suggests that genetics is a key factor (Tick et al., 2016), previous studies have estimated that non-heritable factors account for >50% of the neurobiology of ASD (Mayer et al., 2014).

Traffic-related air pollutants such as particulate matter (PM) and nitrogen dioxide (NO_2) have been suggested as environmental risk factors

Abbreviations: ASD, autism spectrum disorder; PM, particulate matter; NO_2, nitrogen dioxide; SCQ, social communication questionnaire; OTU, operational taxonomic unit; DAG, directed acyclic graph; IQR, interquartile range; RDA, redundancy analysis.

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for ASD (Dutheil et al., 2021; Volk et al., 2013). While NO_2 is mainly emitted from automobile exhaust and combustion of fossil fuels (Shang et al., 2020), PM is a mixture of toxic substances with various particle sizes and chemical properties, including sulfates, nitrates, ammonia, black carbon, dust, polycyclic aromatic hydrocarbons, metallic carbon, and volatile organic compounds (Zhang et al., 2021). Both air pollutants show high annual exposure levels in South Korea and are under active regulation by the Korean government. In 2019, the annual mean PM_{10} and NO_2 levels (42 and 52.6 μg/m^3) in Seoul, the capital of South Korea, were higher than in metropolitan cities such as Los Angeles (29 and 43.2 μg/m^3), Tokyo (16 and 26.3 μg/m^3) and London (18 and 32 μg/m^3) (airkorea.or.kr, http://www.epa.gov, http://www.kankyo.metro.tokyo.jp, http://uk-air.defra.gov.uk). Although results on the association between traffic-related air pollution and ASD have been inconsistent, previous research has suggested that PM with an aerodynamic diameter ≤10 μm (PM_{10}) and NO_2 are related to an increased risk of ASD (Flores-Pajot et al., 2016; Chen et al., 2018; Wang et al., 2021). However, research on the mechanism underlying the association between air pollution and autistic traits is scarce.

Many individuals with ASD report comorbid gastrointestinal symptoms—constipation, abdominal pain, diarrhea, gas, and vomiting (Vuong and Hsiao, 2017)—as well as deficient gut epithelium integrity and increased intestinal permeability (Emanuele et al., 2010). The gut microbiota regulates central nervous system activities through various pathways (Liu et al., 2019a), such as regulating the hypothalamic–pituitary–adrenal axis (Sudo, 2012) and producing short-chain fatty acids (SCFA) that affect brain function (Ray, 2017). A previous meta-analysis found dysbiotic microbial compositions in children with ASD (Iglesias-Vázquez et al., 2020); however, a distinct microbial signature for ASD has not been defined yet (Vuong and Hsiao, 2017).

Air pollution exposure can alter the composition of the gut microbiome (Bailey et al., 2020). Mucociliary clearage of inhaled air pollutants in the lung and contaminated food/drinking water are major routes that PM enters the gastrointestinal tract (Salim et al., 2014). PM can either support or inhibit the growth of specific microbes, causing alteration in the composition and function of the gut microbiota (Gao et al., 2017; Korpela et al., 2019; Adams et al., 2015). Moreover, $PM_{2.5}$ and PM_1 exposures showed negative associations with alpha diversity indices and the relative abundance of most *Firmicutes*, *Proteobacteria*, and *Verrucomicrobia* bacteria (Liu et al., 2019b). NO_2 was associated with alternation in the gut microbiome profile in young adults, including increased *Firmicutes* abundance at the phylum level and *Coriobacteriaceae*, *Ruminococcaceae*, and *Adidobacteriaceae* abundance at the family level (Fouladi et al., 2020).

The microbiome is associated with both air pollution and autistic traits; however, this complex relationship has not been investigated yet. Furthermore, it can potentially mediate environmental risk factors in ASD (Vuong and Hsiao, 2017). The microbiota has bi-directional relationships with both genetics and environment; host genetics affect its composition and function, while environmental factors, including age, infections, diet, and xenobiotics, further shape the microbial profile (Falony et al., 2016). Moreover, early-life alterations in the microbiota can have long-term consequences for health and disease (Kumar et al., 2014). This study aimed to examine whether pre- and postnatal PM_{10} and NO_2 exposures impact autistic traits at 6 years of age through the alteration of the gut microbiome among the children in an ongoing birth cohort. It also aimed to explore the association of PM_{10} and NO_2 exposure (1st, 2nd, and 3rd trimesters of pregnancy; ages 2, 4, and 6 years) with autistic traits at age 6 years, the relationship of PM_{10} and NO_2 exposure with the gut microbiome composition at age 6 years, and the association between microbiome profiles and autistic traits. Mediation analyses of statistically significant findings were also conducted to confirm the "air pollutant exposure–gut microbiome–autistic traits" pathway.

2. Methods and materials

2.1. Study design and participants

Data from an ongoing prospective cohort study—the Environment and Development of Children (EDC) study—were used (Kim et al., 2018). From the 726 pregnant women recruited from hospitals in Seoul, the capital city of South Korea, and two nearby regions (Incheon and Kyoung-gi) from August 2008 to July 2010, we collected information on the mothers' socio-demographical characteristics during the second trimester of pregnancy (between 14 and 27 weeks of gestation). We then contacted the mothers and enrolled 425 children of the mothers at age 2 and additionally 301 at age 4 at the Seoul National University Hospital, Seoul, South Korea. Their children were followed up every 2 years; 425, 645, and 574 children at age 2, 4 and 6 years, respectively. At age 6, we started to collect one fecal sample from each child in 2016 and analyzed the gut microbiome of 173 randomly selected children, due to limited budget, out of the 243 children who were not exposed to antibiotics at the time of sample collection. After excluding those with missing air pollution data or autistic trait scores, 170 of the children were included in the prenatal exposure analyses. For postnatal exposure analyses, 132 children with information on air pollution exposure levels at all 3 ages (age 2, 4, and 6 years) were included. The sample size for prenatal exposure analyses and childhood exposure analyses for the *Verrucomicrobia* was 108 and 84, after excluding the participants with zero relative abundance for *Verrucomicrobia*. For comparison, we explored the relation between air pollution and autistic traits in the main cohort after excluding those with missing data (n = 568).

Informed consent was obtained from all guardians. The study protocol was reviewed by the Institutional Review Board of Seoul National University Hospital (IRB No. 1201-010-392) and followed the principles of the Declaration of Helsinki.

2.2. Estimation of PM_{10} and NO_2 exposure levels

Air pollution exposure levels were extracted from air quality monitoring data recorded by 300 air quality monitoring systems of the Ministry of Environment (Seoul, South Korea: https://www.airkorea.or.kr). Air pollutant concentrations, including PM_{10} (in micrograms per cubic meter μg/m^3) and NO_2 (in μg/m^3) were recorded by the hour. The mean was calculated using 75% of the contributing values. Finally, 24-h mean concentrations were calculated for each monitoring site. According to the participants' addresses, individuals were linked to the air pollution levels measured at the nearest monitoring station based on Euclidean distance (between 100 m and 10 km) using ArcGIS (version 10.1; ESRI Inc., Redlands, CA, USA). Levels of PM_{10} and NO_2 exposure during pregnancy (1st, 2nd, and 3rd trimester) and annual residential levels at ages 2, 4, and 6 years were estimated.

2.3. Assessment of autistic traits

We used the parent-rated Social Communication Questionnaire (SCQ) at age 6 years to quantify autistic traits. The SCQ is a 40-item questionnaire that evaluates ASD symptoms like communication abilities, social skills, and repetitive behaviors during the previous 3 months. The first item asks about minimal verbal skills, and the sum of the remaining 39 binary items (1: yes, 0: no) equals the total SCQ score (Snow, 2013). Individuals with higher scores were considered more autistic.

2.4. 16s rRNA sequencing

Fecal material of the participants was collected at age 6 years and frozen at −80 °C until DNA extraction with a DNeasyPowerSoil Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions, and quantification using Quant-IT PicoGreen (Invitrogen,

Waltham, MA, USA).

The sequencing libraries were processed using the Illumina 16S Metagenomic Sequencing Library protocols to amplify the V3 and V4 regions (Supplemental Methods).

2.5. Operational taxonomic unit (OTU) analysis

We formed the original library and single long reads by assembling paired-end sequences generated sequencing both directions of the library with FLASH (v1.2.11) (Magoč and Salzberg, 2011). (Supplemental Methods). Quality control data was presented in Table S1.

The alpha diversity of the microbiome (Chao, Shannon, and Inverted Simpson index) was calculated to evaluate species diversity and evenness using the "phyloseq" package of R version 4.0.2 (The Comprehensive R Archive Network, Vienna, Austria; http://cran-r-project.org). We excluded rare phyla, which were not commonly detected in the children, such as Cyanobacteria, Fusobacteria, Syndergistetes, and Tenericutes (detection rates were 2.3%, 13.7%, 0.6%, and 4.0%, respectively). In the main anlaysis, we examined four phyla that were detected in all participants (n = 170): *Actinobacteria*, *Bacteriodetes*, *Proteobacteria*, and *Firmicutes*. In addition, we examined Verrucomicrobia that was detected among 108 and 84 children in the prenatal exposure and postnatal exposure analyses, respectively. The $\log_2$-transformed values of the relative abundances were used to normalize the phylum distribution (Fig. S1).

2.6. Definition of covariates

The list of potential covariates was created after review of previous literature (Kim et al., 2021; Yi et al., 2021). The potential covariates were maternal age at pregnancy (years), maternal education ($<$ or $\geq$ college education), family income status (monthly family income $<$ or $\geq$ $3500), diabetes mellitus (DM) during pregnancy (yes or no), pre-pregnancy body mass index (BMI, kg/m^2), child's age (in months), sex, multiple gestation birth (singleton or twin/triplet), child's BMI, birth order ($<$ or $\geq$ 2nd), delivery mode (vaginal delivery or cesarean section), prematurity ($<$ or $\geq$ 37 weeks), low birth weight ($<$ or $\geq$ 2.5 kg), breastfeeding status (exclusive breastfeeding, mixed feeding, or exclusive formula feeding), and season of birth (spring, summer, autumn, or winter). Different covariates were selected for the prenatal and postnatal air pollution analyses. We excluded potential mediators for the prenatal analyses, which were DM during pregnancy, prematurity, and low birth weight. Some potential covariates, including breastfeeding, delivery mode, child's BMI, and season of birth, could not have confounded prenatal air pollutant exposure levels and were only addressed in postnatal analyses. Based on exploratory analyses, potential covariates that were related to SCQ scores, air pollutant levels, or relative abundances of phyla were found (Table S2, S3, S4, and S5). The following final covariates were selected based on the definition of confounders: variables associated with both exposure and outcome, but are not in the causal pathway between exposure and outcome (Hernán et al., 2002). We depicted the relationships between variables by building a data-driven directed acyclic graph (DAG; http://www.dagitty.net/) based on the statistical associations between the involved variables and potential covariates (Fig. S2): age, sex, multiple gestation births, and family income for the prenatal models and age, sex, multiple gestation births, family income, the season of birth, low birth weight, delivery mode, and breastfeeding for the postnatal models.

2.7. Statistical analysis

Pearson correlation coefficients for the correlations between PM_{10} and NO_2 levels during pregnancy and childhood were calculated. The differences in PM_{10} and NO_2 over time were investigated using intraclass correlation coefficients (ICCs; two-way mixed models, single rater, absolute agreement option: ICC(3,1)).

We compared the characteristics of the main (n = 568) and subset cohorts (n = 170) using independent t-tests (for continuous variables) or chi-square tests (for categorical variables).

Due to the right-skewness of SCQ scores at age 6 years (Fig. S3), we implemented Poisson regression for subsequent analyses. The associations between potential covariates and SCQ scores were explored with univariate Poisson regression models. The association between covariates and air pollutant exposure levels and between covariates and phyla relative abundance were examined in linear regression models.

2.8. Association between air pollution and SCQ scores

The association between PM_{10} or NO_2 exposure in each exposure period and SCQ scores at age 6 years was examined using multivariable Poisson regression models in the main (n = 568) and subset (n = 170 for prenatal, n = 132 for postnatal) cohorts. The statistically significant associations were visualized using smoothing splines. As we assumed a Poisson distribution of SCQ scores using a log-link function, the risk of higher SCQ scores associated with an interquartile range (IQR) increase in air pollution level was expressed as a percent change (%) using the following formula: (e^(β*IQR)-1)*100%, where β was an estimate from the Poisson regression model, and IQR is interquartile range of air pollution.

2.9. Associations between air pollution and microbiome profile

The association of air pollutants with alpha diversity indices was explored using multivariable linear regression. Levels of PM_{10} and NO_2 were included as explanatory variables in the redundancy analysis (RDA) computed squared-root-transformed unweighted UniFrac distances, conducted by the "vegan" package of R. We estimated the significance of variation in the microbiome data explained by explanatory variables by the Monte Carlo permutation test (1000 permutations). We used partial RDA models to determine the amount of variation in microbiome community composition explained solely by PM_{10} or NO_2 exposure after controlling for covariates. The associations between exposure to air pollutants and the relative abundance of the four phyla were also tested using multiple linear regression models. As the relative abundance was $\log_2$-transformed, the risk of higher relative abundance associated with an IQR increase in air pollution level was expressed as a percent change (%) using the following formula: (2^(β*IQR)-1)*100%, where β was an estimate from the regression model, and IQR is interquartile range of air pollution.

2.10. Association between microbiome profile and SCQ scores

The associations of the alpha diversity indices/relative abundance of the phyla found statistically significant in the aforementioned analyses and SCQ scores were examined using multivariable Poisson regression.

2.11. Mediation analyses

The "air pollution–microbiome–autistic traits" pathway was tested in cases where all three pairs of associations among air pollution exposure, microbiome, and SCQ scores were statistically significant. We tested the indirect association between air pollution and autistic traits through changes in the microbiome composition by using nonparametric estimation model-based mediation analyses. A predetermined pathway was established, in which air pollution influences a mediator (gut microbiome), which then affects autistic traits. No unmeasured confounding or effect modification was anticipated among the included components. The proportion mediation represents the average amount of indirect association between air pollution and autistic traits via changes in the microbiome composition relative to the average total association.

For comparison, mediation models were constructed for various time windows of exposure (1st, 2nd, and 3rd trimester of pregnancy, age at 2,

4, 6 years) to air pollutants (PM_{10} and NO_2) and microbiota at phyla levels (*Actinobacteria*, *Bacteriodetes*, *Proteobacteria*, *Firmicutes*, and *Verrucomicrobia*). As the microbiome profile and SCQ scores were measured cross-sectionally, the different direction of mediation effects (i.e., air pollution–autistic traits–gut microbiome) was examined. The "mediation" package in R was used to precise p-values of the estimates of the total, direct, and median effects using nonparametric bootstrapping with 20,000 simulations (Tingley et al., 2014).

All statistical analyses were performed using IBM SPSS Statistics for Windows version 22 (IBM Corp., Armonk, N.Y., USA) and R version 4.0.2. Statistical significance was defined as $p < 0.05$ (two-tailed).

3. Results

3.1. General characteristics of the participants

The characteristics of the participants in the main (n = 568) and subset (n = 170) cohorts were similar (Table 1). In the subset cohort, the mean maternal age at pregnancy was 31.4 ± 3.5 years. The majority of mothers were college graduates (85.9%) and most children came from higher-income families (71.8%). Regarding the children's characteristics, 51.2% were boys, 32.4% were born by cesarean section, and 5.3% were born with a low birth weight or prematurely.

Table 1
Characteristics of the participants in the main and subset cohort at age 6.

Characteristics	Variables	Main cohort (n = 568)	Subset cohort (n = 170)	P-value
Maternal	Maternal age at pregnancy, years, mean (SD)	31.4 (3.6)	31.4 (3.5)	0.99
	Maternal Education, N (%)			0.48
	< College education	92 (16.2)	24 (14.1)	
	≥ College graduate	476 (83.8)	146 (85.9)	
	Monthly household income, N (%)			0.63
	< $3500, N (%)	173 (30.5)	48 (28.2)	
	≥$3500, N (%)	395 (69.5)	122 (71.8)	
	Smoking during pregnancy, N (%)			
	Non-smoker	554 (100)	151 (100)	
	Smoker	0 (0)	0 (0)	
	DM during pregnancy, yes, N (%)	22 (3.9)	9 (5.3)	0.42
	Prepregnancy BMI, mean (SD)	20.9 (2.7)	20.8 (2.3)	0.63
Child	Sex, boys, N (%)	296 (52.1)	87 (51.2)	0.86
	BMI, kg/m^2, mean (SD)	15.8 (1.8)	15.6 (1.7)	0.19
	Season of birth, N (%)			**0.03**
	Spring	146 (25.7)	33 (19.4)	
	Summer	175 (30.8)	51 (30.0)	
	Autumn	163 (28.7)	45 (26.5)	
	Winter	84 (14.8)	41 (24.1)	
	Delivery mode, N (%)			0.36
	Vaginal delivery	362 (63.7)	115 (67.6)	
	Cesarean section	206 (36.3)	55 (32.4)	
	Low birth weight, yes, N (%)	40 (7.0)	9 (5.3)	0.42
	Prematurity, yes, N (%)	44 (7.7)	9 (5.3)	0.28
	Breastfeeding			0.37
	Exclusive breastfeeding	172 (30.3)	58 (34.1)	
	Mixed feeding	374 (65.8)	109 (64.1)	
	Formula feeding	20 (3.5)	3 (1.8)	
	Twin, yes, N (%)	50 (8.8)	15 (8.8)	0.99
	Birth order, first child, N (%)	325 (57.2)	94 (55.3)	0.66
	SCQ score, age 6, mean (SD)	3.6 (2.7)	3.4 (2.9)	0.42

Abbreviations: SD, standard deviation; DM, diabetes mellitus; BMI, body mass index; SCQ, social communication questionnaire.
P-value for difference of characteristics between main and subset cohort (chi-square test or Fisher's exact test for categorical variables and *t*-test for continuous variables).

3.2. PM_{10} and NO_2 exposure levels

The distribution of PM_{10} and NO_2 levels at exposure periods is presented in Table S6. The mean levels of PM_{10} exposure were 55.5 ± 10.3, 54.1 ± 11.7, 53.8 ± 12.8, 54.5 ± 7.4, 46.0 ± 4.7, 50.7 ± 5.6, and 49.2 ± 5.2 μg/m^3 during the 1st, 2nd, 3rd, and 1–3rd trimester of pregnancy, and at age 2, 4, and 6 years, respectively. The mean exposure levels for NO_2 were 63.2 ± 9.5, 62.0 ± 11.2, 61.6 ± 11.4, 62.2 ± 7.9, 58.3 ± 7.7, 60.2 ± 7.0, and 54.9 ± 7.5 μg/m^3, during the 1st, 2nd, 3rd, 1–3rd trimester of pregnancy, and at age 2, 4, and 6 years, respectively. The ICC for PM_{10} was 0.91 for the trimesters of pregnancy and 0.46 for age 2–6 years. The ICC for NO_2 was 0.38 for the trimesters of pregnancy and 0.38 for age 2–6 years. The correlation coefficients of both PM_{10} pairs (0.3 and 0.41 for pregnancy, 0.65 and 0.55 for childhood) and NO_2 (0.5 and 0.53 for pregnancy, 0.39 and 0.55 for childhood) for adjacent periods were small to moderate. There were moderate and weak correlations between PM_{10} and NO_2 measured in the same pregnancy trimesters (range 0.48–0.60) and during childhood (range −0.14–0.06), respectively (Fig. S4).

3.3. Association between PM_{10} and NO_2 exposure and autistic traits

The association between PM_{10} and NO_2 exposure and SCQ scores at age 6 years are shown in Table 2 for the subset cohort (n = 170 and n = 108) and Table S7 for the main cohort (n = 568). In the subset cohort (n = 170), an IQR increase of PM_{10} exposure during the 1st trimester and at ages 2 and 4 years was associated with increased SCQ scores at 6 years of age (10.6% change, 95% confidence interval [CI]: 1.1, 21.0; 16.8% change, 95% CI: 2.3, 33.3; 15.7% change, 95% CI: 1.9, 31.4, respectively; Fig. S5). When the sample size was reduced to n = 108, only PM_{10} exposure at age 4 was associated with SCQ scores at age 6 (19.1% change, 95% CI: 1.4, 40.1), and NO2 exposure during the 1st trimester of pregnancy was associated with increased SCQ scores at age 6 (18.3% change, 95% CI: 4.4, 34.1). Similarly, in the main cohort, the exposure windows that showed statistically significant associations between PM_{10} exposure and SCQ scores were the 1st trimester, and the age 2 and 4 periods (9.4% change, 95% CI: 4.4, 14.5; 14.6% change, 95% CI: 6.3, 23.6; 17.4% change, 95% CI: 8.1, 27.6). The effect sizes of associations were similar in the subset and main cohorts.

In the subset cohort, NO_2 exposure during the 1st trimester was statistically significantly associated with increased SCQ scores at age 6 years (12.1% change per IQR increase, 95% CI: 0.1, 25.5). However, in the main cohort, NO_2 exposure was not related to increased SCQ scores in any exposure windows. Rather, NO_2 exposure at ages 4 and 6 years was negatively associated with SCQ scores at age 6 years (−8.8% change, 95% CI: −13.6, −3.7; −6.6% change, 95% CI: −12.6, −0.1).

3.4. Association between PM_{10} or NO_2 exposure and gut microbiome

The range of alpha diversity indices according to sex is shown in Fig. S6. The distribution of the relative phyla abundance is presented in Fig. S7, which shows that *Bacteroides* was the most dominant phylum, followed by *Firmicutes*, *Actinobacteria*, and *Proteobacteria and Verrucomicrobia*. Considering alpha indices, only NO_2 exposure during the 3rd trimester was associated with the Chao index at age 6 years (4.9 increase per IQR increase, 95% CI: 0.14, 9.67; Table S8). In the RDA analysis, PM_{10} exposure during the 1st trimester showed a statistically significant association with the composition variation of the gut microbiome at age 6 years (R^2 = 1.6%, p = 0.03; R^2 = 1.4%, p = 0.01 for the order and family level, respectively; Fig. 1, Table S9).

When examining the phylum level, PM_{10} exposure during the 1st trimester was associated with increased *Proteobacteria* abundance (66.9% increase per IQR increase of PM_{10}, 95% CI: 21.3, 129.8). PM_{10}

Table 2
Associations between exposure to air pollution and SCQ scores at age 6, according to exposure windows.

Pollutant and Exposure windows	Crude[a] (n = 170)		Adjusted[b] (n = 170)		Crude[a] (n = 108)		Adjusted[b] (n = 108)	
	(% change [95% CI])[c]	p-value	(% change [95% CI])[c]	p-value	(% change [95% CI])[c]	p-value	(% change [95% CI])[c]	p-value
PM_{10}								
1st trimester[d]	**11.9 (2.4, 22.1)**	**0.01**	**10.6 (1.1, 21.0)**	**0.03**	4.6 (−6.5, 17.0)	0.40	5.8 (−5.6, 18.5)	0.37
2nd trimester[d]	3.0 (−7.4, 14.6)	0.59	−1.5 (−12.2, 10.6)	0.73	−5.7 (−17.2, 7.3)	0.40	−11.1 (−22.8, 2.4)	0.11
3rd trimester[d]	6.3 (−7.2, 21.9)	0.31	4.2 (−9.1, 19.4)	0.47	0.8 (−15.9, 20.8)	0.93	−2.0 (−18.6, 17.9)	0.83
1st-3rd trimesters[d]	**11.1 (1.7, 21.4)**	**0.02**	8.4 (−1.3, 19.1)	0.09	2.5 (−8.2, 14.3)	0.65	0.1 (−11.0, 12.6)	0.98
Age 2[d]	**16.0 (3.5, 30.1)**	**0.01**	**16.8 (2.3, 33.3)**	**0.03**	**19.7 (3.7, 38.2)**	**0.01**	13.9 (−79.2, 524.3)	0.14
Age 4[d]	**18.3 (5.0, 33.2)**	**<0.01**	**15.7 (1.9, 31.4)**	**0.02**	**27.2 (9.5, 47.9)**	**<0.01**	**19.1 (1.4, 40.1)**	**0.03**
Age 6[d]	4.2 (−10.5, 21.5)	0.62	7.8 (−8.3, 26.6)	0.38	7.6 (−11.7, 31.0)	0.46	0.8 (−18.0, 24.0)	0.93
NO_2								
1st trimester[d]	**15.0 (2.9, 28.5)**	**0.01**	**12.1 (0.1, 25.5)**	**0.04**	**19.6 (5.8, 35.3)**	**<0.01**	**18.3 (4.4, 34.1)**	**0.01**
2nd trimester[d]	6.2 (−15.4, 33.3)	0.60	−3.2 (−14.9, 10.1)	0.57	6.1 (−7.6, 21.8)	0.43	−1.5 (−15.2, 14.5)	0.87
3rd trimester[d]	17.2 (−5.5, 5.4)	0.15	4.8 (−6.7, 17.7)	0.40	17.8 (−2.8, 42.9)	0.11	10.8 (−9.0, 34.9)	0.29
1st-3rd trimesters[d]	**26.9 (1.9, 58.0)**	**0.03**	3.1 (−4.5, 22.3)	0.20	**15.7 (3.7, 29.1)**	**0.01**	12.0 (−0.2, 25.8)	0.06
Age 2[d]	11.2 (−4.7, 29.6)	0.18	4.9 (−4.2, 14.9)	0.27	10.4 (−1.4, 23.6)	0.09	11.1 (−2.3, 26.4)	0.11
Age 4[d]	**−17.2 (-29.6, -2.6)**	**0.02**	−7.6 (−15.6, 1.1)	0.10	**−17.9 (-26.9, -7.8)**	**<0.01**	−10.4 (−21.4, 2.2)	0.10
Age 6[d]	3.1 (−15.0, 25.1)	0.75	2.6 (−7.9, 14.3)	0.59	−4.9 (−16.1, 7.8)	0.44	−0.8 (−12.9, 13.0)	0.90

Abbreviations: PM_{10}, particulate matter with an aerodynamic diameter ≤10 μm; NO_2, nitrogen dioxide; SCQ, social communication questionnaire; CI, confidence interval; IQR, interquartile range.
Adjusted for child's age, sex, twin, family income, season of birth, low birthweight, delivery mode and breastfeeding for exposure windows during childhood.
[a] Adjusted for age and sex.
[b] Adjusted for child's age, sex, twin, family income for exposure windows during pregnancy.
[c] Per IQR increase; Statistically significant results shown in bold.
[d] Sample size: n = 170 for pregnancy exposure (1st – 3rd trimesters), n = 132 for childhood exposure (age 2–6).

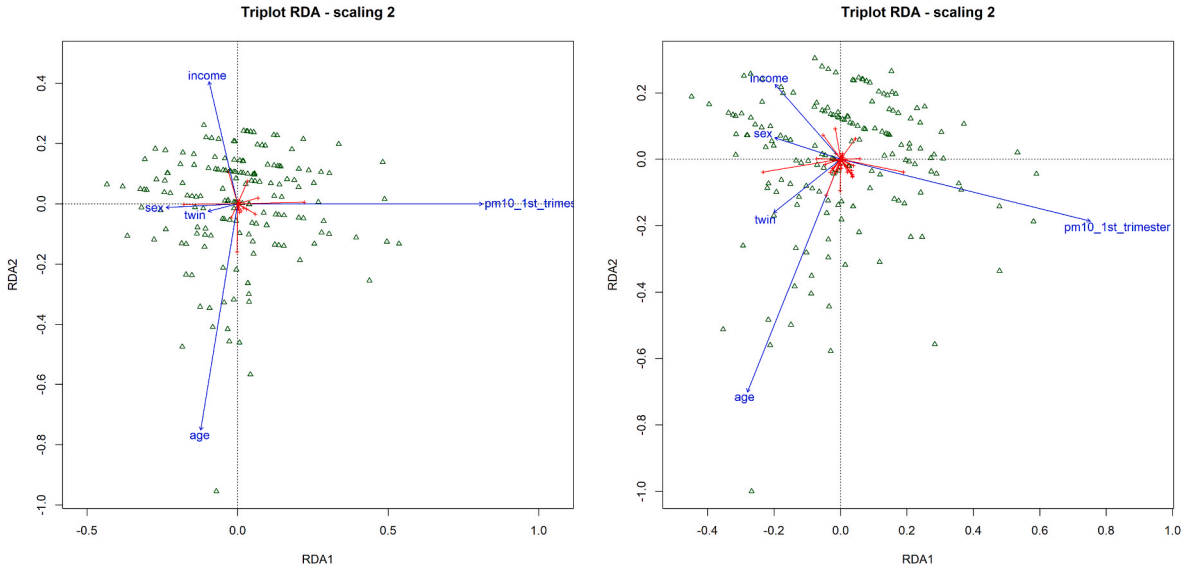

Fig. 1. RDA analysis on the association between PM_{10} exposure in the 1st trimester of pregnancy and distribution of gut microbiome (order level and family level), (a) A 3.2% of the total variance was explained by the model. The first and second axes explained 1.7% and 0.9% of the variance, (b) A 3.5% of the total variance was explained by the model. The first and second axes explained 1.8% and 0.9% of the variance., Abbreviations: RDA, redundancy analysis; PM_{10}, particulate matter with an aerodynamic diameter ≤10 μm; NO_2, nitrogen dioxide.

exposure at age 2 years was also related to an increase in *Proteobacteria* relative abundance (74.1% change, 95% CI: 6.0, 188.6; Table 3). The association between NO_2 exposure during the 1st trimester and *Proteobacteria* relative abundance was marginally statistically significant (48.1% change, 95% CI: −0.1, 119.6). NO_2 exposure at age 2 years was associated with a decrease in *Proteobacteria* relative abundance (−29.2% change, 95% CI: −47.2, −5.0). NO_2 exposure at age 2 was associated with increased *Bacteroidetes* relative abundance at age 6 (21.6% increase per IQR increase of NO_2, 95% CI: 4.1, 42.0). PM_{10} exposure during the 1st trimester was associated with decreased *Verrucomicrobia* abundance (45.4% decrease per IQR increase of PM_{10}, 95% CI: −67.5, −8.4).

3.5. Association between gut microbiome and autistic traits

The relative abundance of *Proteobacteria* was statistically significantly associated with SCQ scores (4.4% change per 2-fold increase in relative abundance, 95% CI: 1.3, 7.5) in the prenatal model (n = 170). However, there was no statistically significant relationship between *Proteobacteria* relative abundance and SCQ scores in the postnatal exposure group (n = 132; Table 4).

Table 3
Associations between exposure to air pollution and relative abundance at the phylum level, by exposure windows.

Exposure windows	*Bacteroidetes*[a]		*Actinobacteria*[a]		*Proteobacteria*[a]		*Firmicutes*[a]		*Verrucomicrobia*[a]	
	((% change [95% CI])[b]	p-value	(% change [95% CI])[b]	p-value	(% change [95% CI])[b]	p-value	(% change [95% CI])[b]	p-value	(% change [95% CI])[b]	p-value
PM_{10}										
1st trimester*	−13.7 (−29.2, 5.2)	0.15	−1.5 (−20.4, 21.8)	0.88	**66.9 (21.3, 129.8)**	**<0.01**	3.2 (−4.4, 11.3)	0.45	**−45.4 (-67.5, -8.4)**	**0.02**
2nd trimester*	−15.0 (−43.5, 10.1)	0.22	22.6 (−7.3, 62.2)	0.16	25.1 (−17.7, 90.3)	0.29	2.1 (−9.5, 15.1)	0.73	−32.1 (−64.2, 28.6)	0.24
3rd trimester*	8.9 (−19.8, 47.9)	0.60	4.4 (−25.3, 45.8)	0.81	−10.7 (−45.9, 47.3)	0.63	−2.8 (−13.0, 8.7)	0.59	−9.5 (−60.7, 108.8)	0.80
1-3 trimesters*	−8.6 (−26.6, 13.9)	0.41	7.6 (−14.6, 35.5)	0.55	38.5 (−1.5, 94.8)	0.06	0.6 (−7.9, 9.8)	0.89	−34.4 (−61.3, 11.3)	0.12
Age 2*	−11.7 (−33.1, 16.6)	0.39	2.2 (−27.7, 44.3)	0.91	**74.1 (6.0, 188.6)**	**0.03**	2.6 (−8.8, 15.5)	0.69	−30.0 (−69.3, 59.8)	0.40
Age 4*	3.1 (−20.3, 33.4)	0.83	−14.5, (−37.8, 17.4)	0.33	58.5 (−0.6, 152.6)	0.06	0.1 (−10.6, 12.1)	0.99	−13.6 (−60.2, 87.2)	0.72
Age 6*	16.1 (−16.3, 61.1)	0.37	1.2 (−32.6, 51.8)	0.96	31.8 (−27.5, 139.6)	0.36	−3.4 (−16.6, 11.9)	0.64	−15.9 (−68.5, 124.4)	0.73
NO_2										
1st trimester*	−14.5 (−33.9, 10.5)	0.21	−17.5 (−37.3, 8.5)	0.17	48.1 (−0.1, 119.6)	0.05	5.8 (−4.3, 16.9)	0.28	15.0 (−40.0, 120.4)	0.68
2nd trimester*	−16.6 (−37.6, 11.4)	0.24	−3.4 (−29.3, 32.0)	0.84	−1.1 (−38.1, 57.9)	0.97	8.3 (−3.1, 21.0)	0.21	7.1 (−49.8, 128.4)	0.86
3rd trimester*	11.4 (−13.6, 43.7)	0.41	−15.9 (−36.1, 10.8)	0.21	−22.0 (−49.0, 19.1)	0.24	−1.1 (−11.0, 10.0)	0.85	16.3 (−41.0, 129.2)	0.66
1-3 trimesters*	−10.2 (−31.6, 17.8)	0.43	−16.9 (−38.5, 12.4)	0.23	3.9 (−32.9, 60.9)	0.85	6.3 (−4.3, 18.2)	0.29	16.6 (−42.6, 136.9)	0.67
Age 2*	**21.6 (4.1, 42.0)**	**0.02**	−6.8 (−24.0, 14.2)	0.49	**−29.2 (-47.2, -5.0)**	**0.02**	−0.8 (−7.8, 6.7)	0.83	−4.5 (−42.4, 58.4)	0.86
Age 4*	11.6 (−7.6, 34.7)	0.24	−0.9 (−21.5, 25.1)	0.92	−13.2 (−38.3, 22.0)	0.41	7.1 (−1.2, 16.1)	0.11	40.3 (−21.0, 149.0)	0.25
Age 6*	18.6 (−3.6, 46.0)	0.11	−11.6 (−31.5, 13.9)	0.33	−15.2 (−41.4, 22.7)	0.38	0.6 (−8.3, 10.3)	0.87	20.7 (−35.3, 125.3)	0.56

*Sample size: n = 170 for pregnancy exposure, n = 132 for childhood exposure (age 2–6) for *Bacteroidetes, Actinobacteria, Proteobacteria* and *Firmicutes*. n = 108 for pregnancy exposure, n = 84 for childhood exposure (age 2–6) for *Verrucomicrobia*.
Abbreviations: PM_{10}, particulate matter with an aerodynamic diameter ≤10 μm; NO_2, nitrogen dioxide; CI, confidence interval; IQR, interquartile range.
Adjusted for child's age, sex, twin, family income, season of birth, low birthweight, delivery mode and breastfeeding for exposure windows during childhood.
[a] Adjusted for child's age, sex, twin, family income for exposure windows during pregnancy.
[b] Per IQR increase; Statistically significant results shown in bold.

Table 4
Associations between gut microbiome and SCQ scores at age 6.

Microbiome	Crude[a]		Adjusted[b]	
	(% change [95% CI])[c]	p-value	(% change [95% CI])[c]	p-value
Proteobacteria (n = 170)	**4.7 (1.7, 7.9)**	**<0.01**	**4.4 (1.3, 7.5)**	**<0.01**
Proteobacteria (n = 132)	**4.3 (0.9, 7.8)**	**0.01**	2.6 (−26.6, 43.5)	0.13

Abbreviations: SCQ, social communication questionnaire; CI, confidence interval.
Adjusted for child's age, sex, twin, family income, season of birth, low birthweight, delivery mode and breastfeeding for n = 132 sample.
[a] Adjusted for age and sex.
[b] Adjusted for child's age, sex, twin, family income for exposure windows for n = 170 sample.
[c] Per 1-unit increase for *Bacteroidetes*, per 2-fold increase for *Proteobacteria*; Statistically significant results shown in bold.

3.6. Mediation effect of microbiome on the association between air pollution and autistic traits

In the mediation analysis of *Proteobacteria* relative abundance for the association between PM_{10} exposure during the 1st trimester and SCQ scores at age 6 years, both the indirect and direct effects were statistically significant, indicating that the association between PM_{10} exposure during the 1st trimester and autistic traits was partially mediated by changes in *Proteobacteria* relative abundance (mediation proportion: 25.1%, p = 0.01). The mediation analysis also showed that a marginally significant proportion of the association between NO_2 exposure during the 1st trimester and autistic traits is attributed to changes in *Proteobacteria* relative abundance (proportion mediated: 16.5%, p = 0.06; Fig. 2 and Table 5).

3.7. Comparison with other mediation models

There were no other mediation models wherein the indirect, direct, and total effects were all statistically significant, nor were there models wherein the mediated proportion was statistically significant (Table S10). No other time window of air pollution exposure showed significant mediation. The indirect effect of *Proteobacteria* abundance on the association between PM_{10} exposure at age 2 years and SCQ scores at age 6 years was marginally significant (proportion mediated: 13.5%, p = 0.07). *Proteobacteria* abundance also marginally mediated the association between PM_{10} exposure at age 4 years and SCQ scores at age 6 years (proportion mediated: 15.3%, p = 0.06). In other models, the indirect effect of *Proteobacteria* abundance on the association between NO_2 at age 2 years and SCQ at age 6 years was statistically significant (−4.9% change per IQR increase, 95% CI: −9.5, −0.001); however, the association showed a negative direction, in contrast to the direct effect (15.7% change, 95% CI: 5.8, 26.5). Therefore, the total effect was not statistically significant (10.1% change, 95% CI: −4.5, 27.0).

When the mediation effects of autistic traits on the association between air pollution (PM_{10} or NO_2) levels during the 1st trimester of pregnancy and *Proteobacteria* abundance were examined, the indirect effect was no longer statistically significant, and the proportions

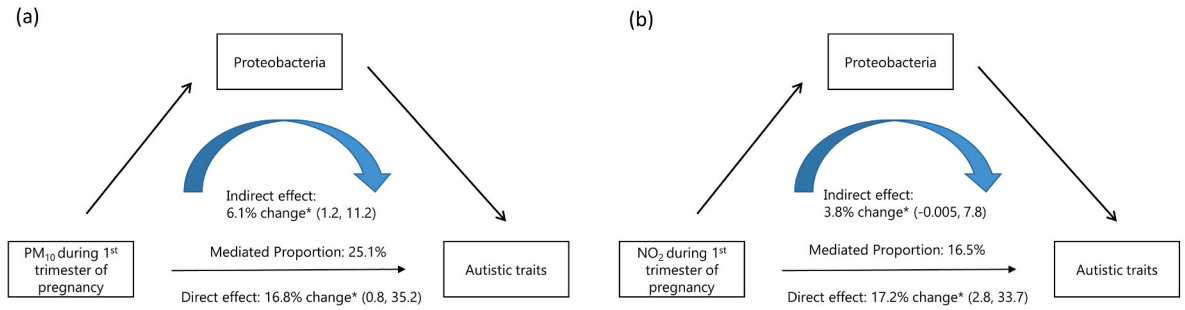

Fig. 2. Mediation analysis of the association between air pollution and autistic traits through gut microbiome changes, (a) Mediation of *Proteobacteria* relative abundance on the association between PM_{10} during the1st trimester and autistic traits, (b) Mediation of *Proteobacteria* relative abundance on the association between NO_2 during the1st trimester and autistic traits, *: % change per interquartile range, Abbreviations: PM_{10}, particulate matter with an aerodynamic diameter ≤10 μm; NO_2, nitrogen dioxide.

Table 5
Mediating role of proteobacteria on the association between air pollution and SCQ scores.

Path	Indirect effect		Direct effect		Total effect		Mediated proportion (%)	p-value
	% change [95% CI])[a]	p-value	% change [95% CI])[a]	p-value	% change [95% CI]))[a]	p-value		
1st trimester PM_{10} – proteo – SCQ	**6.1 (1.2, 11.2)**	**<0.01**	**16.8 (0.8, 35.2)**	**0.04**	**23.8 (10.6, 38.7)**	**<0.01**	25.1	**0.01**
1st trimester NO_2 – proteo – SCQ	3.8 (−0.005, 7.8)	0.05	**17.2 (2.8, 33.7)**	**0.03**	**21.7 (9.4, 35.3)**	**0.01**	16.5	0.06

Abbreviations: SCQ, social communication questionnaire; CI, confidence interval; PM_{10}, particulate matter with an aerodynamic diameter ≤10 μm; NO_2, nitrogen dioxide; IQR, interquartile range, statistically significant results shown in bold.
Sample size: n = 170 and n = 132 for the 1st trimester and age 2 analyses, respectively.
[a] Per IQR-increase in air pollutant exposure.

mediated were 3.1% and 11.0%, respectively (p-values > 0.05; Table S11).

4. Discussion

To our knowledge, this study is the first to investigate the mediating role of the microbiome in the association between environmental toxins and autistic traits. Interestingly, the microbiota profile, specifically *Proteobacteria* at the phylum level, showed 25.1% and 16.5% mediation effects on the associations between both 1st trimester PM_{10} and NO_2 exposure, respectively, and autistic traits in children. These findings suggest a plausible mechanism underlying the relation between air pollution and ASD and add evidence to the existing literature on the gut–brain axis suggested in ASD.

Despite the proposed role of the microbiome as a mediator of genetic and environmental risk factors, research has been scarce, specifically on ASD. The majority of earlier evidence comes from animal studies that indirectly investigated the effect of the microbiome. For example, oral treatment of *B. fragilis* or *B. thetalotaomicron* to the offspring of the rodent model of maternal immune activation, a model that resembles infection during pregnancy, improved the gut microbial composition and permeability while reducing ASD-related behavior (Hsiao et al., 2013; Kentner et al., 2019). Similarly, modeling maternal exposure to valproic acid, an anticonvulsant drug associated with an increased risk for ASD (C.G. de Theije et al., 2014), resulting in offspring with altered gut microbiota composition, neuro-inflammation, and ASD-associated behavioral abnormalities (C.G.M. de Theije et al., 2014). Meanwhile, two human studies have investigated the mediating role of the microbiome linking air pollution with liver function or glucose levels (Yi et al., 2021; Alderete et al., 2018); however, this study is the first study to have autistic traits as the outcome variable.

Prenatal (1st trimester of pregnancy) and postnatal (at age 2 and 4 years) exposure to air pollutants was associated with autistic traits at age 6 years, suggesting long-lasting effects. According to the Developmental Origins of Health and Disease, exposure to environmental agents results in long-lasting human physiology and behavior alterations (Barker, 2007). These long-term effects may be partly due to the developing epigenetic code (Suter et al., 2010, 2013) and microbiome (Chu et al., 2016; Chu and Aagaard, 2016), since the gut microbiome can be transferred across the placenta during fetal development (Braniste et al., 2014; Jašarević et al., 2016), leading to varied effects in the offspring. Moreover, alterations in the maternal microbiome due to environmental risk exposure can be passed on, since mammals acquire their initial microbiome via birthing. The microbiota may convey lasting effects on health and disease through the epigenetic modification of the host genome (Kumar et al., 2014; Cortese et al., 2016). Moreover, epigenetic changes caused by microbiota can influence host transcriptional patterns. For example, fatty acid butyrate—a SCFA produced by the microbiome—can inhibit the action of histone deacetylase inhibitor, and result in disruption in cell cycle progression, gene silencing, differentiation, and genotoxic reactions (Waldecker et al., 2008). As this study did not include methylation data, further studies that incorporate both methylome and microbiome data could provide an integrated multi-omics description on the pathway linking air pollution and autistic traits.

The 1st trimester of pregnancy as well as ages 2 and 4 years were susceptible exposure periods to the neurotoxic effects of PM_{10}. These exposure periods have been suggested to be susceptible to environmental risk factors in previous studies. Brain development most rapidly occurs during series of time-sensitive periods when neuroplasticity is heightened (Ismail et al., 2017; Meredith, 2015). The early fetal period is marked by prominent neurogenesis, the 2nd trimester of pregnancy and first 2 years of early life is characterized by synaptogenesis (Johnston et al., 2009), while ages 2 to 10 is the period when synaptic pruning rapidly occurs (Huttenlocher and Dabholkar, 1997). The susceptible periods of air pollution exposure related to autistic traits overlap with the critical periods of neurodevelopment, which also coincide with the developmental periods of the gut microbiome. Gut microbiome changes appear to occur most dynamically during the first 3 years of life (Yatsunenko et al., 2012; Derrien et al., 2019). Therefore, it can be

speculated that the microbiome would be more prone to environmentally toxic materials up to age 3, when changes are more rapid. However, only microbiome data at age 6 years were obtained, and thus, it is unclear in which period our participants' microbiome was most susceptible. Considering the relative stability of the gut microbiome after the age of 3 years, the microbiome profile at age 6 years might reflect that of an earlier stage. However, recent studies have also found differences in the gut microbiome of 7–12-year-olds compared to adults, suggesting that complete maturation of the gut microbiome may take longer than previously suggested (Zhong et al., 2019). Thus, further studies using microbiome data from periods of early life are warranted to confirm this study's hypothesis.

Proterobacteria abundance was associated with both PM_{10} and NO_2 exposure during the 1st trimester of pregnancy. Exposure to mixed vehicle emissions increased the abundance of lung *Proterobacteria* in mice (Daniel et al., 2021). Exposure to $PM_{2.5}$ was associated with increased *Proterobacteria* abundance in buccal mucosa microbacteria (Wu et al., 2021). A previous study involving adult patients with schizophrenia found that NO_2 exposure and PM_{10} in the preceding year explained 3.7% and 7.5% of the gut microbiome composition, respectively (Yi et al., 2021). Another study reported that NO_2 explained 4.4% of the variance in gut microbiome composition in overweight to obese adults (Fouladi et al., 2020). In our study, 1st trimester PM_{10} and NO_2 explained 1.6% and 0.7% of the gut microbiome at age 6 years, respectively, which is a smaller effect size compared to previous studies. However, direct comparison between these studies is not recommended since this study investigated children's microbiota profiles, whereas previous studies targeted adult populations. Moreover, the distribution and concentration of air pollutants from different countries or regions may vary.

Proterobacteria abundance was cross-sectionally associated with autistic traits at age 6 years. Although previous studies on microbiota differences in patients with ASD have been highly heterogeneous, most studies found that the overall microbiota composition of ASD cases differs from that of controls (Bundgaard-Nielsen et al., 2020). However, there were no specific bacteria consistently associated with ASD diagnosis or severity. Nevertheless, *Proteobacteria* abundance was found to be elevated in individuals with ASD compared with controls (Finegold et al., 2010; Williams et al., 2011). *Proteobacteria* is associated with host inflammation (Shin et al., 2015) and produces a potent toxic factor lipopolysaccharide (Liu et al., 2019a); exposure to this factor can reduce glutathione in the brain (Zhu et al., 2007; Chauhan and Chauhan, 2006), suggesting possible neurotoxic effects.

Among the Organization for Economic Cooperation and Development member countries, South Korea ranked first in terms of mean population exposure to $PM_{2.5}$ in 2019 (Lee et al., 2018), as most cities in South Korea were urbanized (Shin et al., 2022). The new air quality guideline by the World Health Organization (WHO) recommends that the annual mean PM_{10} value should not exceed 15 $\mu g/m^3$ and that the annual standard NO_2 concentration should not exceed 10 $\mu g/m^3$ (World Health Organization, 2021). The air quality guideline by the Ministry of Environment in South Korea has set higher limit levels: 50 $\mu g/m^3$ for the annual PM_{10} value and 0.03 ppm (56.4 $\mu g/m^3$) for the annual NO_2 value (Kumbhakar et al., 2021). As the mean concentrations of these pollutants in the present study were similar to the recommended values mentioned in the South Korea guideline and higher than those mentioned in the WHO guideline and neurotoxicity of air pollutants was observed at these values, we suggest a stricter policy to regulate air pollution levels.

This study has some limitations. We used data from a community-based cohort and none had undergone formal testing for ASD. Although the SCQ has a valid and reliable questionnaire (Corsello et al., 2007), its primary purpose is screening for ASD. Therefore, further studies using diagnostic interviews are needed to expand the results to clinical populations. As the relationship between the gut microbiome and autistic traits was cross-sectional, causal relations are not definite, and reverse causation is possible. We did not provide refined information such as genera or species, due to the lower detection rates compared to the phlya and substantial reduction in sample size.. Moreover, the small sample size limits the results' statistical power, and further replication in a larger population is warranted. We did not adjust for multiple testing and rather focused on the trend of the results; thus, the findings of this study should be interpreted cautiously. Although various covariates were accounted for, some confounding factors, including diet diversity and other endocrine-disrupting chemicals, were not considered (Yap et al., 2021). Furthermore, other individual data that may affect exposure to air pollution, such as indoor air pollution, physical activity, time spent outdoors, and occupational status, were lacking (Lim et al., 2021). Air pollutant exposure was obtained through data from monitoring stations and exposure misclassification cannot be ruled out. Moreover, we did not consider changes in exposure levels over time among individuals when we investigated exposure windows. This problem may not influence our study results significantly as we compared exposure levels within a 3-year period (e.g., exposure years in 2015–2017 for children at age 6), where exposure levels may not change significantly in the three years. Furthermore, we did not consider other air pollutants such as ultrafine particles, which may have greater capacity to reach the gut and brain (Akimoto, 2003; Oberdorster et al., 1994), due to lacking data. In addition, other air pollutants, including carbon monoxide, ozone and $PM_{2.5}$, were not included in the analyses as these pollutants were not associated with autistic scores in the study (Fig. S8). Lastly, the majority of participants resided in urban areas, and most were from highly educated and high income families; thus the results may not be generalizable to populations in rural regions.

Despite these limitations, this study was strengthened by its longitudinal design and repetitive assessment of $PM_{10,}$ NO_2 exposure and autistic traits. We identified multiple susceptible periods in early life and explored the mediating role of the gut microbiome in bridging environmental toxins and neurodevelopmental outcomes.

5. Conclusions

Air pollution during the 1st trimester of pregnancy may affect autistic traits at age 6 years through the alteration of *Proteobacteria* abundance. Future studies with larger sample sizes and microbiome samples at earlier ages are warranted. Moreover, research on whether correction of gut microbial dysbiosis could reduce the impact of PM_{10} exposure on autistic traits is needed.

Institutional review board statement

Informed consent was provided by all guardians. The study protocol was reviewed by the Institutional Review Board of Seoul National University Hospital (IRB No. 1201-010-392) and followed the principles of the Declaration of Helsinki.

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Declaration of competing interest

The authors declare they have no actual or potential competing financial interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114047.

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