Environmental Research 228 (2023) 115795

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Evidence for an association of prenatal exposure to particulate matter with clinical severity of Autism Spectrum Disorder

João Xavier Santos [a,b], Pedro Sampaio [a,b], Célia Rasga [a,b], Hugo Martiniano [a,b], Clarissa Faria [c], Cátia Café [c,d], Alexandra Oliveira [c,d], Frederico Duque [c,d], Guiomar Oliveira [c,d], Lisete Sousa [e], Ana Nunes [b,f], Astrid Moura Vicente [a,b,*]

[a] Instituto Nacional de Saúde Doutor Ricardo Jorge, Avenida Padre Cruz, 1649-016, Lisboa, Portugal
[b] BioISI - Biosystems & Integrative Sciences Institute, University of Lisboa, Faculty of Sciences, Campo Grande, C8, 1749-016, Lisboa, Portugal
[c] Unidade de Neurodesenvolvimento e Autismo, Serviço Do Centro de Desenvolvimento da Criança, Centro de Investigação e Formação Clínica, Hospital Pediátrico, Centro Hospitalar e Universitário de Coimbra, Coimbra, Portugal
[d] Faculty of Medicine, University Clinic of Pediatrics and Coimbra Institute for Biomedical Imaging and Translational Research, University of Coimbra, Coimbra, Portugal
[e] Departamento de Estatística e Investigação Operacional e Centro de Estatística e Aplicações, Faculdade de Ciências, Universidade de Lisboa, Lisbon, Portugal
[f] Departamento de Física, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal

ARTICLE INFO

Handling Editor: Jose L Domingo

Keywords:
Air pollution
Autism spectrum disorder
Clinical severity
Early-life exposure
Particulate matter
Pregnancy

ABSTRACT

Early-life exposure to air pollutants, including ozone (O_3), particulate matter ($PM_{2.5}$ or PM_{10}, depending on diameter of particles), nitrogen dioxide (NO_2) and sulfur dioxide (SO_2) has been suggested to contribute to the etiology of Autism Spectrum Disorder (ASD). In this study, we used air quality monitoring data to examine whether mothers of children with ASD were exposed to high levels of air pollutants during critical periods of pregnancy, and if higher exposure levels may lead to a higher clinical severity in their offspring. We used public data from the Portuguese Environment Agency to estimate exposure to these pollutants during the first, second and third trimesters of pregnancy, full pregnancy and first year of life of the child, for 217 subjects with ASD born between 2003 and 2016. These subjects were stratified in two subgroups according to clinical severity, as defined by the Autism Diagnostic Observational Schedule (ADOS). For all time periods, the average levels of $PM_{2.5}$, PM_{10} and NO_2 to which the subjects were exposed were within the admissible levels defined by the European Union. However, a fraction of these subjects showed exposure to levels of $PM_{2.5}$ and PM_{10} above the admissible threshold. A higher clinical severity was associated with higher exposure to $PM_{2.5}$ ($p = 0.001$), NO_2 ($p = 0.011$) and PM_{10} ($p = 0.041$) during the first trimester of pregnancy, when compared with milder clinical severity. After logistic regression, associations with higher clinical severity were identified for $PM_{2.5}$ exposure during the first trimester ($p = 0.002$; OR = 1.14, 95%CI: 1.05–1.23) and full pregnancy ($p = 0.04$; OR = 1.07, 95%CI: 1.00–1.15) and for PM_{10} ($p = 0.02$; OR = 1.07, 95%CI: 1.01–1.14) exposure during the third trimester. Exposure to PM is known to elicit neuropathological mechanisms associated with ASD, including neuroinflammation, mitochondrial disruptions, oxidative stress and epigenetic changes. These results offer new insights on the impact of early-life exposure to PM in ASD clinical severity.

1. Introduction

Autism Spectrum Disorder (ASD) is a clinically heterogeneous neurodevelopmental disorder characterized by two core symptoms that are usually evident during infancy and early childhood: 1) deficits in social communication and interaction; 2) repetitive sensory-motor behaviors and restricted interests (Hof et al., 2021; Lord et al., 2020, American Psychiatric Association, 2013). The disorder has a lifelong impact in the affected individuals, and significant family and societal burdens (van Heijst et al., 2015).

* Corresponding author. Avenida Padre Cruz, 1649-016, Lisboa, Portugal.
E-mail addresses: joao.xavier@insa.min-saude.pt (J.X. Santos), pedro.sampaio@insa.min-saude.pt (P. Sampaio), celia.rasga@insa.min-saude.pt (C. Rasga), hugo.martiniano@insa.min-saude.pt (H. Martiniano), fariacp2@yahoo.com.br (C. Faria), alexandraoliveira@chuc.min-saude.pt (A. Oliveira), fredericoduque.chuc@gmail.com (F. Duque), guiomar@chuc.min-saude.pt (G. Oliveira), lmsousa@fc.ul.pt (L. Sousa), amnunes@fc.ul.pt (A. Nunes), astrid.vicente@insa.min-saude.pt (A.M. Vicente).

https://doi.org/10.1016/j.envres.2023.115795
Received 29 July 2022; Received in revised form 6 March 2023; Accepted 28 March 2023
Available online 5 April 2023

Despite the massive efforts for the discovery of genetic biomarkers in the last decades, a genetic etiology is only found in up to 40% of the cases (Genovese and Butler, 2020), highlighting the complex etiologic architecture underlying ASD (Rylaarsdam and Guemez-Gamboa, 2019). Heritability estimates obtained in family studies range from 50 to 84%, (Sandin et al., 2017; Bai et al., 2019; Pettersson et al., 2019), suggesting that environmental risk factors have an important contribution to ASD risk. Currently, the origin of ASD is considered multifactorial, with multiple genes and early exposure to environmental factors contributing to reach a liability threshold for disease expression (Rylaarsdam and Guemez-Gamboa, 2019; Cheroni et al., 2020; Santos et al., 2022).

ASD pathophysiology results from brain reorganization, which may be due to altered cortical development, disrupted neural circuitry and/or synaptic dysfunction (Torre-Ubieta et al., 2016; Manoli and State, 2021). Given the early onset of ASD, environmental risk factors are expected to exert their effect early in development, particularly during the prenatal and early postnatal periods, spanning the preconception period to the first years of life (Lyall et al., 2014). However, critical time periods when environmental risk factors may affect brain functioning are still debated. Previous studies provided strong evidence for an association of maternal exposure to ubiquitous neurotoxins, namely airborne pollutants, with ASD (Rossignol et al., 2014; Modabbernia et al., 2017; Santos et al., 2021). Positive associations between ASD risk and *in utero* exposure to nitrogen dioxide (NO_2) (Becerra et al., 2013; Volk et al., 2013; Volk et al., 2014; Kerin et al., 2018; Jung et al., 2013; Raz et al., 2018) and to particulate matter (PM, classified as $PM_{2.5}$ or PM_{10} depending to their aerodynamic diameter) (Volk et al., 2013; Volk et al., 2014; Raz et al., 2015; Talbott et al., 2015; Geng et al., 2019; Kaufman et al., 2019) are well documented. Early exposure to sulfur dioxide (SO_2) (Jung et al. 2013) and ozone (O_3) (Kim et al., 2017; Jung et al., 2013) has also been implicated in ASD risk.

In many world regions, including the European Union, PM, NO_2, SO_2 and O_3 constitute criteria air pollutants, for which permissible levels of exposure have been set by government agencies responsible for public health and environmental policies (Sicard et al., 2021). Thus, assessment of exposure to these criteria air pollutants is obligatory, and assured through air quality monitoring networks composed by monitoring stations that permanently measure concentrations of ambient air pollutants (Guerreiro et al., 2014). Georeferencing strategies, which leverage surveillance data collected and stored by these monitoring networks, are a valuable tool to investigate associations between air pollution exposure and disease risk or severity (Fleury et al., 2021; Gaio et al., 2022).

The objectives of the present study were two-fold: to understand if mothers of children with ASD show evidence of exposure to the selected air pollutants above admissible levels, and whether exposure to these air pollutants during the prenatal period and the first year of the child's life is associated with clinical severity of the disease. Because air pollution compounds can cross the placenta and blood-brain barrier potentially leading to neurodevelopmental problems in the offspring (Costa et al., 2020; Ghazi et al., 2021; Zhu et al., 2021), maternal exposure is an adequate proxy to estimate fetal exposure. This approach is justified by the clinically heterogeneous character of the disorder, and the absence of universal objective, quantitative and validated biomarkers for ASD (McPartland et al., 2020), with stratification being an adequate approach to identify risk factors specific to clinical subgroups (Frye et al., 2019) and eventually to develop effective preventive and treatment strategies.

2. Methods

2.1. Participants and clinical assessment

Participants were identified from an ongoing collaboration between National Institute of Health Doutor Ricardo Jorge and Centro Hospitalar e Universitário de Coimbra (CHUC). Clinical assessment of participants was carried out by trained clinicians from the Autism Clinic, which is the reference clinic for autism at CHUC, the main pediatric hospital for children in Região Centro of Portugal. This hospital is reference for neurodevelopmental disorders, and children from across this region are referred here by general practitioners and pediatricians when there are concerns regarding their development and behavior. ASD cases were assessed using the Autism Diagnostic Interview-Revised (ADI-R) (Lord et al., 1994) and the Autism Diagnostic Observation Schedule (ADOS) (Lord et al., 2000), which constitute diagnostic gold standard assessment tools. Diagnosis fulfilled the criteria defined in the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) (American Psychiatry Association). Children also undergo extensive functional and cognitive assessments (Oliveira et al., 2007).

A categorical measure of ASD severity can be derived from the ADOS, an observational, direct assessment of the child, which comprises a series of tasks to evaluate existing autistic behaviors. The ADOS severity metric ranges from 1 to 10, and is calculated using raw scores from the ADOS modules 1–3 (Gotham et al., 2009). This metric allows the categorization of the clinical severity of ASD, with score distribution collapsed into three categories: "Autism" (scores 6 to 10), "Autism Spectrum Disorder" (scores 4 to 5), and "Non-Spectrum" (scores 1 to 3), with "Autism" being the most severe clinical form, while Non-Spectrum includes subjects with subthreshold ASD symptoms. Children in the "Autism" and "Autism Spectrum Disorder" categories were considered for this study. Children in the "Non-Spectrum" ADOS category were excluded because they do not meet the thresholds for ASD diagnosis according to this instrument.

A total of 217 children with ASD, including 18 females and 199 males were included in the study. The higher clinical severity group, with ADOS scores in the category "Autism", included 163 subjects (12 females and 151 males), while the milder clinical severity group, with ADOS scores in the category "Autism Spectrum Disorder", included 54 subjects (6 females and 48 males).

Cognitive assessment was carried out for all children using age-appropriate Wechsler Intelligence Scales, with scores <70 defining Intellectual Disability (ID). Adaptive functioning was assessed using the Vineland Adaptive Behavior Scales (VABS) scores, a standardized measure of adaptive behavior across four domains: communication, daily living skills, socialization and motor skills (the motor skills domain was not considered in this study, because it can only be measured for children older than 5 years old) (Sparrow et al., 2016). VABS global and domain scores ≤ 70 indicate a low adaptive functioning.

All participants were born in Região Centro of Portugal between 2003 and 2016. In 2020, Portugal had a population of nearly 10.3 million inhabitants, of whom 2.2 million (21.4%) resided in Região Centro. This region covers the Portuguese geographic and socioeconomic backgrounds, with both interior and coastal areas as well as rural and urban settings.

Informed consent to participate in the study was obtained for all subjects by their parents/legal guardian. Ethical approval was obtained by Comissão de Ética para a Saúde do Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA, I.P.) and by Comissão de Ética do Centro Hospitalar e Universitário de Coimbra (CE-CHUC).

2.2. Sample characterization

Summary sociodemographic and clinical data were retrieved for the 217 cases. Data on birth weight, gestational age and type of delivery was examined because these variables are predictors of neonatal health and were previously associated with ASD risk (Modabbernia et al., 2017). Birth weight categories were defined in accordance to World Health Organization (WHO) standards, and include Very Low Birth Weight (<1500 g), Low Birth Weight (1500–2499 g), Normal Birth Weight (2500–3999 g) and High Birth Weight (≥ 4000 g) (Cutland et al., 2017). Gestational age categories were also defined according to WHO, and include preterm birth (<37 weeks), full term birth (37–41 weeks), and

post-term birth (>41 weeks). Advanced maternal and paternal ages at birth have also been previously associated with ASD risk (Wu et al., 2017) and were categorized as follows: <25 years old, 26–34 years old, ≥35 years old. A woman pregnant at 35 years old or more is considered to be at higher risk of adverse maternal and perinatal outcomes (Glick et al., 2021), and recent epidemiological studies show that men with 35 years or more also have higher risks of adverse outcomes in the offspring (Fossé et al., 2020; Janeczko et al., 2020). Parental education level, which functions as a proxy for socioeconomic status, has also been associated with ASD risk (Meter et al., 2010). We defined three categories for parental education: illiteracy and primary education, secondary education and tertiary education. Season of birth has also been associated with ASD, and can act as a confounder for an association between exposure to air pollution and ASD risk (Lee et al., 2019). To account for this, we divided the participants in two groups: participants born between October and March and participants born between April and September.

2.3. Air pollution data and estimation of individual pollution exposure

Publicly available data on air quality from 2003 to 2016 was obtained from the Air Quality Database *(QualAr)* web-portal managed by Portuguese Environment Agency (*Agência Portuguesa do Ambiente*; APA). The network to assess air quality in Portugal was created in 2001, and thus we had no data on air quality for children born before 2003 (Ministério do Ambiente e Ordenamento to Ministério do Ambiente e Ordenamento do Território and Direção Geral do Ambiente, 2001; in Portuguese). As legislated by the European Union, air quality monitoring in Portugal is carried out by background stations that measure the levels of criteria air pollutants, including O_3, $PM_{2.5}$, PM_{10}, NO_2, and SO_2. In Região Centro there are 8 background stations distributed by six areas of influence with homogeneous air quality, with each station reflecting the air pollution for all the municipalities located in their area of influence. These areas of influence were delimited by the Portuguese Directorate-General for the Environment, through measurements of the distribution of air pollutants concentrations to assess air quality across Portugal, considering also the location of industrial hotspots, population density, land use and orography. The information was used to establish the current legislation that defines the plan for assessment and management of air quality in Portugal. For a more detailed description regarding the delimitation of areas of influence refer to supplementary material. We did not consider other sources of air pollution, for instance measured by traffic or industrial stations because these are not widely distributed in Região Centro (there are only 2 traffic stations and industrial stations are non-existing). Information for other factors, like wind patterns, altitude or temperature patterns were also not available. The files downloaded from the APA portal contained the hourly levels of each pollutant, measured in $\mu g/m^3$, for the 2003–2016 time period. A data collection efficiency of at least 75% was considered, meaning that when the hourly levels for a given air pollutant for a given station were missing for 25% or more of the 2003–2016 time-period that data was not analyzed. This is a conservative approach that allows us to have higher confidence in the measures by the monitoring stations, since missing data, particularly during continuous time-periods, may reflect an issue with the station.

Using this data, together with information on gestational age for each subject, we retrospectively estimated average hourly levels for O_3, $PM_{2.5}$, PM_{10}, NO_2 and SO_2, for each five time periods: first, second and third trimesters of pregnancy, full pregnancy and the first year of the child's life. Each subject was assigned to the area of influence covering his or her address at birth. Data for all pollutants was available for 179/217 (82.5%) subjects.

Admissible annual average levels for $PM_{2.5}$, PM_{10}, and NO_2 were retrieved from European Air Quality Standards guidelines, legislated by the European Union (EU) under Directive 2008/50/EU. These admissible levels were established to protect human health, particularly for vulnerable populations, as exposure above those levels is detrimental to human health. To understand whether, in any of the years under analysis (2003–2016), any of the background stations measured levels of air pollutants above the admissible levels stipulated by the EU, increasing hazard risk for the population, these values were compared with the average annual air pollutant concentrations.

For full pregnancy and first year of life time periods, we identified the individuals that were exposed to average concentrations above the thresholds defined as admissible by the EU. Admissible average annual pollution levels for O_3 and SO_2 are not provided by the referred directive.

2.4. Mapping

The municipality of birth was considered the georeferencing unit for mapping. All the municipalities of birth were within the areas of influence covered by the monitoring stations. Mapping was done using the free and open source Geographic Information System software QGIS, 3.18.2 Zürich version. The files in shapefile format containing geometric information for Região Centro where provided by Statistics Portugal (*Instituto Nacional de Estatística*; INE) and APA.

2.5. Statistical analyses

For population characterization, chi-square or Fisher tests were performed to compare the qualitative variables between higher and milder severity groups. Significance was considered at $\alpha = 0.05$.

Fisher tests were also performed to compare the numbers of ASD subjects with exposure levels above and below the admissible annual average levels imposed by European Union, for full pregnancy and first year of life time periods.

A non-parametric Kruskal-Wallis test was performed to compare the concentrations of each air pollutant, for the 2003–2016 time period, between all areas of influence. Significance was considered at $\alpha = 0.05$.

A non-parametric Mann-Whitney test for unpaired samples was used to compare the distribution of exposure levels between patients in the higher severity group and patients in the milder severity group. Comparisons were carried out independently for the five time periods. Significance was considered at $\alpha = 0.05$, and the Holm-Bonferroni correction for multiple testing was applied.

We analyzed the association between exposure to multiple air pollutants and clinical severity using logistic regression. Separate full models were computed for each time period, with the clinical severity as the response Bernoulli variable and the levels of exposure to O_3, $PM_{2.5}$, PM_{10}, NO_2 and SO_2 as independent variables. Covariates previously associated with ASD risk were also added to the model, including the subjects' sex, maternal and paternal age at birth (<35 years old vs ≥ 35 years old), maternal and paternal education (higher education vs non-higher education) and season of birth (birth in October–March vs birth in April–September), as previously done by others (Ongono et al., 2022; Rahman et al., 2022). For regression, the response variable was coded as 0 (milder severity group) or 1 (higher severity group). Stepwise backwards selection was applied, using the Akaike Information Criteria (AIC) algorithm, to iteratively remove independent variables and covariates from the model, in order to find the subset of variables in the dataset resulting in the best performing model (named stepwise model). Significance was considered at $\alpha = 0.05$ and odds ratios (OR) with 95% confidence intervals were calculated. The assumption of linearity between the independent variables and the log-odds of the outcome, for each time period, was tested using Box Tidwell test, considering $\alpha = 0.05$. Multicollinearity between variables that remain in the stepwise models was measured, considering a cut-off value of Variation Inflation Factor (VIF) of 5. For logistic regression analyses, only the 179 subjects with data for all pollutants were considered.

All statistical analyses were performed using the open source software R.

3. Results

3.1. Geographical distribution of participants and pollution levels per area of influence

We mapped the geographical distribution of the 217 children with ASD, using their municipality of birth as the geo-referencing unit (Fig. 1). This georeferenced data was then integrated with the areas of influence defined by APA. Região Centro covers a third of the mainland Portuguese territory, with littoral municipalities having more children with ASD when compared to the interior areas (Fig. 1), reflecting the higher population density and urbanization in coastal areas in Portugal.

The average annual concentrations of the analyzed pollutants, in Região Centro, for the 2003–2016 time period were 59.06 μg/m^3 (range: 45.96–67.10 μg/m^3) for O_3, 9.59 μg/m^3 (range: 5.76–19.58 μg/m^3) for $PM_{2.5}$, 21.58 μg/m^3 (range: 15.53–33.63 μg/m^3) for PM_{10}, 2.59 μg/m^3 (0.67–8.24 μg/m^3) for SO_2 and 9.80 μg/m^3 (5.63–16.36 μg/m^3) for NO_2. For $PM_{2.5}$, PM_{10} and NO_2 it is possible to compare these values with the annual admissible limits imposed by the European Union. The annual average concentrations obtained for these three pollutants were below the annual limits defined by the European Union (25 μg/m^3 for $PM_{2.5}$, 40 μg/m^3 for PM_{10} and 40 μg/m^3 for NO_2).

No significant differences in the concentrations of each air pollutant were observed between areas of influence ($p > 0.05$) (Table S1), for the 2003–2016 time period.

Regarding the distribution of participants by clinical severity across the six areas of influence, we note that these are similarly distributed between the defined areas (Table S2).

3.2. Characterization of the population

Baseline characteristics of the study population, including relevant clinical parameters as well as sociodemographic variables, are shown in Table 1.

Age at diagnosis ranged from 1 to 10 years old (mean age at diagnosis = 3.37 years), with 88% of the participants receiving a positive diagnosis until 4 years old.

As expected, higher and milder clinical severity groups differed significantly in some of the clinical parameters. In the higher severity group, 42.3% have ID, compared to 13.0% in the milder severity group ($p < 0.001$). The groups also differed in the adaptive behavior measures, as estimated by VABS. The higher severity group scored significantly lower across all VABS modules when compared to the milder severity group (Table 1). The two groups did not differ in other clinical analyzed parameters, including birth weight, gestational age, or any of the sociodemographic variables, namely sex, paternal and maternal ages at birth, education level and season of birth (Table 1).

3.3. Average levels of exposure to criteria air pollutants

The distribution of the concentrations of each criteria air pollutant to which the mothers of the 217 participants were exposed, for each pregnancy time period, is shown in Fig. 2. The exposure of the child during the first year of life is also shown. Average concentration levels per time period vary between 61.4 ± 12.1 and 62.2 ± 13.3 for O_3, 11.4 ± 7.8 and 12.8 ± 9.0 for $PM_{2.5}$, 21.3 ± 7.8 and 22.2 ± 9.4 for PM_{10}, 2.3 ± 1.2 and 2.4 ± 1.5 for SO_2 and 8.7 ± 4.4 and 9.0 ± 4.5 for NO_2. The average period concentrations for all the pollutants were below the annual admissible limits defined by the European Union (25 μg/m^3 for $PM_{2.5}$, 40 μg/m^3 for PM_{10} and 40 μg/m^3 for NO_2).

However, it is striking that for a fraction of the subjects, exposure to $PM_{2.5}$ and PM_{10} levels was above the admissible limits for the full pregnancy and first year of life time periods (Fig. 2, Table 2). Furthermore, when stratifying by clinical severity, we observed that many subjects for whom levels of exposure were above the admissible limits have a higher clinical severity (Table 2). We found significant differences in the number of subjects with exposure levels to $PM_{2.5}$ above the admissible limits, between the higher and milder clinical severity groups, for the full pregnancy ($p = 0.049$).

3.4. Exposure to air pollutants during early development in subjects with higher vs milder clinical severity

The group of subjects with higher clinical severity had a significantly higher exposure to $PM_{2.5}$ (adjusted $p = 0.001$), NO_2 (adjusted $p = 0.011$) and PM_{10} (adjusted $p = 0.041$), during the first trimester of pregnancy,

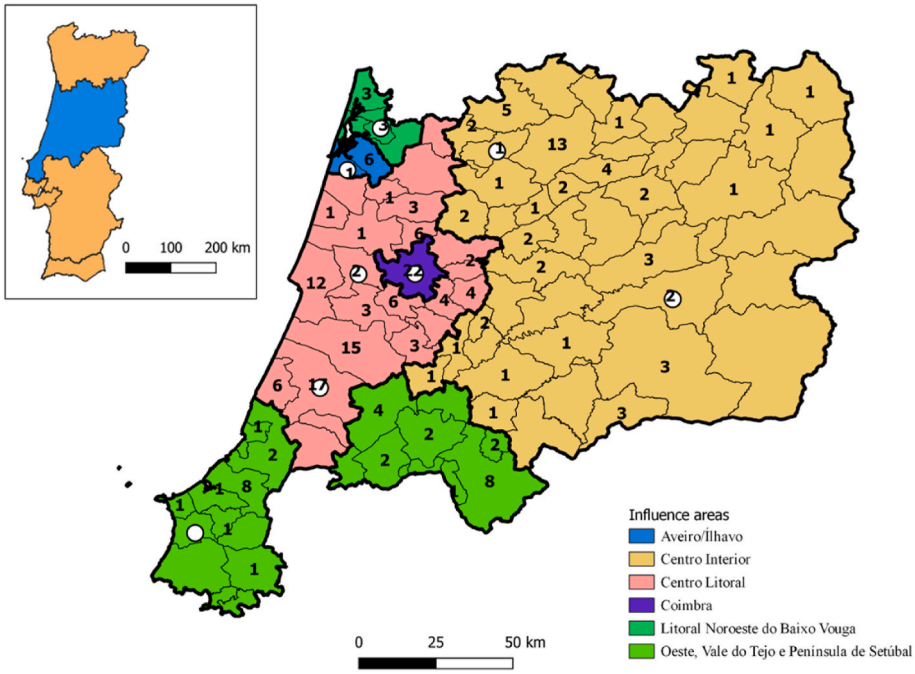

Fig. 1. Geographical distribution of the 217 children by municipality of birth, along Região Centro. Shown are the numbers of children by municipality. The white dots represent the location of air quality monitoring stations. The region is subdivided in six areas of influence defined by APA.

Table 1
Baseline characteristics of the study population.

Variables	Total (n = 217)		Higher severity (n = 163)		Milder severity (n = 54)		
	N	%	n	%	N	%	p-value
Sex							
Male	199	91.7	151	92.6	48	88.9	
Female	18	8.3	12	7.4	6	11.1	0.5612
Intellectual Disability							
With intellectual disability (<70)	76	35.8	69	43.4	7	13.2	
Without intellectual disability (>70)	136	64.2	90	56.6	46	86.8	
Unknown	5		4		1		<0.001*
Vineland Adaptive Behavior Scales							
VABS Global <70	132	67.7	113	75.8	19	41.3	
VABS Global >70	63	32.3	36	24.2	27	58.7	
Unknown	22		14		8		<0.001*
VABS Communication <70	111	56.9	95	63.7	16	34.8	
VABS Communication >70	84	43.1	54	36.3	30	65.2	
Unknown	22		14		8		<0.001*
VABS Daily Living Skills <70	125	64.1	106	71.1	19	41.3	
VABS Daily Living Skills >70	70	35.9	43	28.9	27	58.7	
Unknown	22		14		8		<0.001*
VABS Socialization <70	102	52.3	95	63.7	7	15.2	
VABS Socialization >70	93	47.7	54	36.3	39	84.8	
Unknown	22		14		8		<0.001*
Birth Weight							
<1500 g	8	3.7	6	3.7	2	3.7	
1500–2499 g	14	6.5	9	5.5	5	9.3	
2500–3999 g	189	87.5	143	88.3	46	85.1	
>4000 g	5	2.3	4	2.5	1	1.9	
Unknown	1		1		0		0.809
Gestational age							
<37	23	10.6	18	11.0	5	9.3	
37–41	194	89.4	145	89.0	49	90.7	
>41	0		0		0		0.909
Maternal age at birth							
<25	26	12.2	19	11.8	7	13.0	
26–34	134	62.6	99	61.9	35	64.8	
≥35	54	25.2	42	26.3	12	22.2	
Unknown	3		3		0		0.838
Paternal age at birth							
≤25	14	6.6	8	5.0	6	11.1	
26–34	120	56.1	94	58.8	32	59.3	
≥35	80	37.3	58	36.2	16	29.6	
Unknown	3		3		0		0.244
Maternal educational level							
Illiteracy and Primary education	49	22.6	40	24.5	9	16.7	
Secondary education	89	41.0	62	38.0	27	50.0	
Tertiary education	79	36.4	61	37.4	18	33.3	
Unknown	0		0		0		0.257
Paternal educational level							
Illiteracy and Primary education	85	39.2	64	39.3	21	38.9	
Secondary education	71	32.7	52	31.9	19	35.2	
Tertiary education	61	28.1	47	28.8	14	25.9	
Unknown	0		0		0		0.879
Season of birth							
October–March	112	51.6	83	50.9	29	53.7	
April–September	105	48.4	80	49.1	25	46.3	0.944
Unknown	0		0		0		

Statistical comparison of characteristics between higher (n = 163) and milder (n = 54) severity groups were carried out using Fisher (birth weight) or Chi-square (all other variables) tests, with significance at α = 0.05. Unknown values were not considered for the statistical analyses. HBW – High Birth Weight, LBW – Low Birth Weight, NBW – Normal Birth Weight, VABS – Vineland Adaptive Behavior Scale, VLBW – Very Low Birth Weight; *p-value<0.05.

when compared to the group of subjects with milder severity (Table 3, Table S3). Higher exposure to $PM_{2.5}$ for the whole pregnancy in the group with higher severity was also identified, but was non-significant after correction for multiple testing (10.3 μg/m^3 VS 8.8 μg/m^3, p = 0.036, adjusted p = 0.179) (Table 3, Table S3). No significant differences were identified for the remaining time periods (Table 3).

3.5. Association between exposure to air pollutants during early development and clinical severity

The Box Tidwell test revealed that the linearity assumption between the independent variables and the log odds of the outcome is met, since p > 0.5 for all variables (Table S4).

Summary statistics for stepwise logistic regression models are shown in Table 4 (refer to Table S5 for the results of the full models). The 95% confidence intervals obtained for the logistic regression were not substantially large, indicating that the obtained odds ratios are precise.

The regression model showed that, for exposures occurring during the first trimester of pregnancy, only $PM_{2.5}$ levels had a significant association with higher clinical severity (p = 0.002) (Table 4). The results indicate that per each 1 μg/m^3 $PM_{2.5}$ increment there is a fold increase in the odds of having higher severity by 1.14 (OR = 1.14; 95% CI: 1.05–1.23).

For the second trimester of pregnancy, no significant associations were found (Table 4).

Regarding the third trimester, PM_{10} levels showed a significant association with higher clinical severity (p = 0.02), indicating that per each 1 μg/m^3 PM_{10} increment there is a fold increase in the odds of having a more severe phenotype by 1.07 (95% CI: 1.01–1.14) (Table 4).

When considering the complete pregnancy period, $PM_{2.5}$ levels showed a significant association with higher clinical severity (p = 0.04), indicating that per each 1 μg/m^3 $PM_{2.5}$ increment there is a fold increase in the odds of having higher severity by 1.07 (OR = 1.07; 95% CI: 1.00–1.15) (Table 4).

No significant associations were identified for the period pertaining to the first year of life (Table 4).

Multicollinearity was never found for any independent variable remaining in the stepwise models, which means that no variable can be linearly predicted from the others with a substantial degree of accuracy (Table S6).

Notably, while not significant, sex and maternal education level were included in the stepwise models for all trimesters of pregnancy and first year of life, while sex was also included in the stepwise model for the full pregnancy period. These results suggest that being a female and a lower maternal education contribute to a higher severity of ASD, even when air pollution is the significant variable.

4. Discussion

Ambient air pollution is a major public health issue, responsible for 4.2 million premature deaths globally in 2016, of which 286,000 were children under 15 years old (WHO 2021). In 2005, the World Health Organization (WHO) established expert-based guidelines to lessen the health impact of exposure criteria air pollutants (WHO 2005). Growing evidence supports a role for air pollution in neurodevelopmental problems, including ASD (Volk et al., 2021). In this study, we retrospectively analyzed the exposure of mothers of ASD children to five criteria air pollutants during critical periods of pregnancy, examined whether they were exposed beyond admissible levels at early stages of development, and explored for associations with the offspring clinical severity. We analyzed a dataset of 217 children with ASD, for five well-defined time periods critical for a healthy neurodevelopment (first, second and third trimesters of pregnancy, full pregnancy and first year of life). All these time periods are included in the first 1000 days concept, which refers to the time spanning conception until the second birthday (Moore et al., 2017). This is a period of extreme vulnerability to external influences,

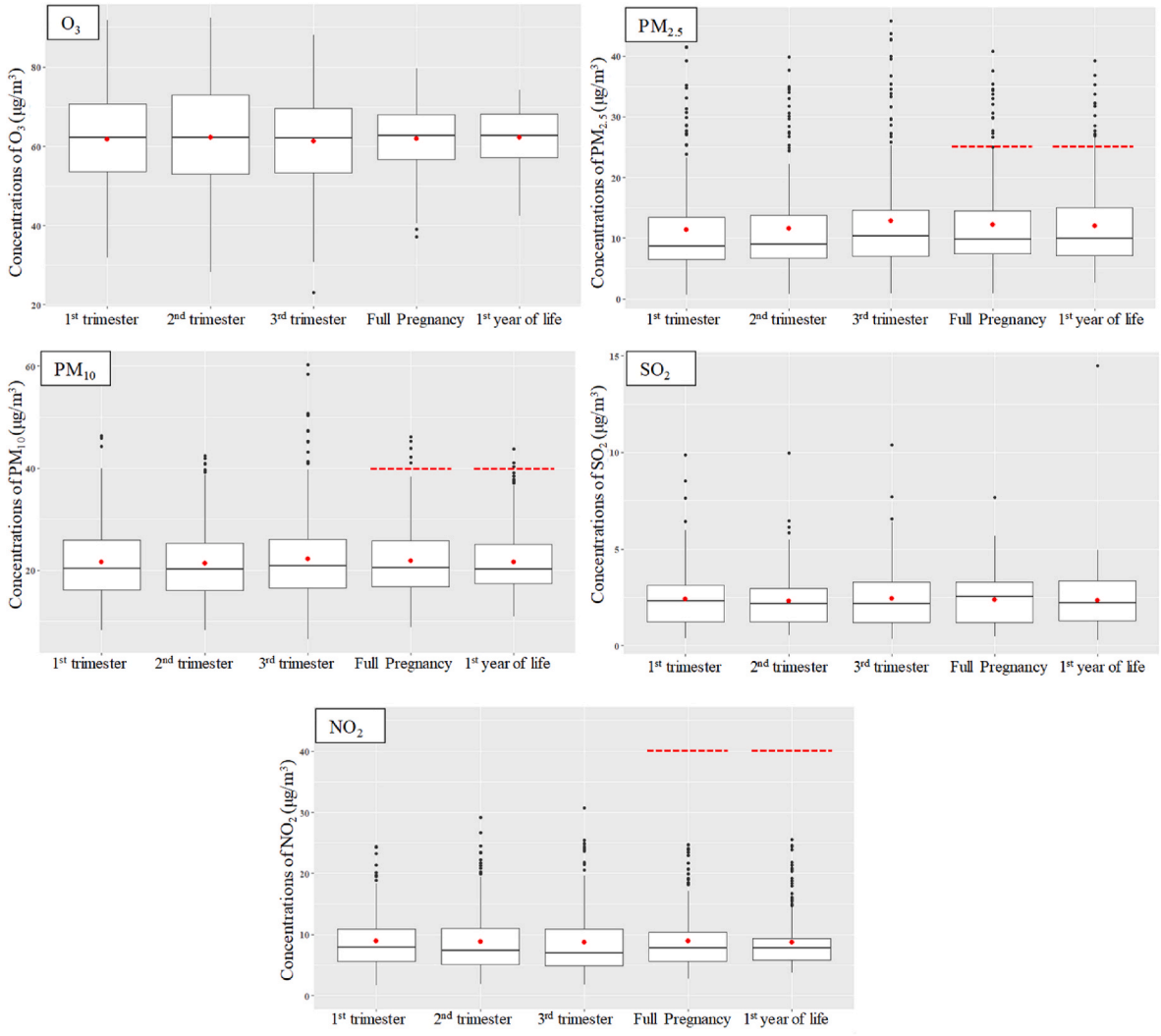

Fig. 2. Boxplot graphs showing the distribution of concentrations of each criteria air pollutant to which the mothers of the 217 participants with ASD were exposed for each pregnancy period, as well as the exposure of the child for the first year of life. Red dots represent average concentrations. The dashed red lines represent annual limit averages imposed by the European Union.

Table 2
Summary statistics regarding the comparison of the numbers of ASD subjects with exposure levels above and below the admissible annual average levels imposed by European Union, for full pregnancy and first year of life time periods.

		$PM_{2.5}$			PM_{10}		
	Severity group	Exposure above admissible levels n (%)	Exposure below admissible levels n (%)	p-value	Exposure above admissible levels n (%)	Exposure below admissible levels n (%)	p-value
Full pregnancy	Higher	17 (10.4%)	146 (89.6%)	0.049*	5 (3.1%)	158 (96.9%)	n/a
	Milder	1 (1.9%)	53 (98.1%)		0 (0)	54 (100%)	
First year of life	Higher	15 (9.2%)	143 (90.8%)	0.252	3 (1.8%)	160 (98.2%)	n/a
	Milder	2 (3.7%)	52 (96.3%)		0 (0)	54 (100%)	

Shown are the p-values for Fisher test between higher and milder clinical severity groups. *p-value<0.05.

where the foundations for an optimal neurodevelopment, and overall lifelong health and wellbeing, are established (Moore et al., 2017).

For the 2003–2016 time period, the average annual concentrations of $PM_{2.5}$, PM_{10} and NO_2 in Região Centro were below the admissible levels imposed by the European Union. This indicates that, for this region, air quality standards are generally fulfilled, as has been reported by other authors (Monteiro et al., 2007, 2015). The average concentrations of $PM_{2.5}$, PM_{10} and NO_2 to which the 217 ASD were exposed during pregnancy and the first year of life, in any of the time periods considered, also met air quality requisites. However, an important fraction of these subjects were individually exposed to concentrations of $PM_{2.5}$ and PM_{10} above the admissible limits, particularly during pregnancy and first year of life. Many of these ASD subjects had a more severe clinical phenotype, and the difference was significant for those whose mothers were exposed to $PM_{2.5}$ and PM_{10} above admissible levels during full pregnancy. This compelled us to explore the possible association between air pollution exposure during defined early developmental time periods and ASD severity.

This work provides novel evidence for an impact of prenatal exposure to $PM_{2.5}$ during the first trimester of pregnancy and a more severe

Table 3
Summary statistics regarding Mann-Whitney test for comparisons of exposure levels to the different air pollutants, in each time-period, for higher vs milder severity groups.

		O_3			$PM_{2.5}$			PM_{10}			SO_2			NO_2		
	Severity group	n	median exposure ($\mu g/m^3$) ±SD	p-value	n	median exposure ($\mu g/m^3$) ±SD	p-value	n	median exposure ($\mu g/m^3$) ±SD	p-value	n	median exposure ($\mu g/m^3$) ±SD	p-value	n	median exposure ($\mu g/m^3$) ±SD	p-value
First Trimester	Higher	162	62.0 ± 12.3	0.270	142	10 ± 8.1	0.001*	162	21.2 ± 8.4	0.041*	155	2.1 ± 1.6	0.866	154	8.5 ± 4.8	0.011*
	Milder	52	64.8 ± 11.0		49	7.4 ± 4.2		52	18.3 ± 4.8		49	2.5 ± 1.3		51	6.9 ± 3.2	
Second Trimester	Higher	163	63.4 ± 13.0	0.799	143	9.2 ± 8.1	0.674	162	20.0 ± 8.1	1	157	2.1 ± 1.3	1	154	7.6 ± 5.6	0.799
	Milder	53	60.1 ± 4.0		50	8.4 ± 5.2		52	20.9 ± 6.5		49	2.4 ± 1.3		51	7.0 ± 3.8	
Third Trimester	Higher	163	62.6 ± 12.2	0.870	143	10.8 ± 9.5	0.870	162	21.6 ± 9.8	1	157	2.2 ± 1.6	1	154	6.9 ± 5.8	0.870
	Milder	53	59.7 ± 10.9		50	8.5 ± 7.2		52	20.7 ± 8.0		49	2.3 ± 1.4		51	7.6 ± 4.6	
Full pregnancy	Higher	163	62.9 ± 8.4	0.939	143	10.3 ± 7.8	0.179	162	20.8 ± 7.9	0.767	157	2.5 ± 1.2	1	154	8.0 ± 4.1	0.767
	Milder	53	60.8 ± 8.4		50	8.8 ± 5.0		52	19.4 ± 5.5		49	2.5 ± 1.2		51	7.7 ± 3.2	
First year of life	Higher	163	62.6 ± 7.3	0.864	143	9.9 ± 7.3	1	162	20.2 ± 7.4	0.864	157	2.1 ± 1.5	1	154	7.9 ± 4.8	0.864
	Milder	53	63.6 ± 6.5		50	9.2 ± 7.2		52	19.9 ± 6.9		49	2.7 ± 1.1		51	7.7 ± 2.8	

Shown are corrected p-values, after Holm-Bonferroni correction for multiple testing. Significance was considered at $\alpha = 0.05$. For uncorrected p-values see Table S2; SD – Standard Deviation.

ASD clinical presentation of the offspring. Our analysis revealed a significant association between a more severe clinical phenotype and prenatal exposure to higher concentrations of this pollutant during the first trimester, when compared to subjects with milder clinical presentations. Logistic regression including several covariates previously associated with ASD risk, further showed that high $PM_{2.5}$ levels are the only variable significantly associated with a more severe phenotype during this time period. Notably, $PM_{2.5}$ levels were also the only significant variable for the full pregnancy, and subjects with higher severity had a significantly higher exposure to this pollutant when compared to subjects with milder severity in this time period, before correction for multiple testing. This trend observed for the full pregnancy is likely driven by the results observed for the first trimester. Previous studies have implicated prenatal exposure to $PM_{2.5}$ in ASD risk (Volk et al., 2013; Volk et al., 2014; Raz et al., 2015; Talbott et al., 2015; Geng et al., 2019; Kaufman et al., 2019). This study reinforces these previous findings and further provide evidence for an impact on the clinical severity of the disorder.

Our findings from the logistic regression also implicate prenatal exposure to higher concentrations of PM_{10} during the third trimester in the modulation of clinical severity increase. This is in agreement with other epidemiologic studies comparing prenatal exposure in subjects with ASD versus neurotypical controls, showing that exposure to PM_{10} during the third trimester of pregnancy was associated with increased risk of ASD (Kalkbrenner et al., 2015; Volk et al., 2013). The biological mechanisms underlying the association of exposure to PM_{10} with clinical severity, specifically in the third trimester and not before, are unclear but may be related with the composition of these particles interfering with neurodevelopmental processes in this period, or with the dynamics of transport across the placental and/or BBB of these larger particles.

Among the analyzed criteria pollutants, PM have the most widespread effects in human health, including neurological outcomes (Anderson et al., 2012; Kim et al., 2020; Werder et al., 2021), in line with our findings of an association between $PM_{2.5}$ and PM_{10} and ASD clinical severity. PM composition varies widely between regions depending on local emission sources, but it generally consists of a complex mixture of solid particles and liquid droplets, containing organic chemicals, acids, metals, black carbon and dust and soil particles.

The stronger associations found for $PM_{2.5}$ relative to PM_{10} are biologically plausible since, due to its small size, $PM_{2.5}$ more easily penetrates the lungs and enters the bloodstream, spreading throughout the body (Kim et al., 2020). $PM_{2.5}$ has been shown to have neurotoxic properties (Liu et al., 2015) and various of its constituents, including metals, black carbon and organic chemicals, cross the placenta (Bové et al., 2019; Familari et al., 2019; Zhu et al., 2021) and the blood-brain barrier (Costa et al., 2020), ultimately reaching the developing brain. Exposure to $PM_{2.5}$ induces pathophysiological mechanisms associated with ASD, including neuroinflammation, mitochondrial disruptions and oxidative stress and epigenetic alterations. Among the neuroinflammatory actions promoted by $PM_{2.5}$ exposure are microglial cell activation (Chen et al., 2020, Yau et al., 2021), which disrupts normal development and neuronal connectivity, and increased expression of several cytokines (Pope III et al., 2016; Zhang et al., 2018; Zou et al., 2020). Altered cytokine profiles have been consistently reported in subjects with ASD, including in the neonatal period (Krakowiak et al., 2017; Masi et al., 2017; Eftekharian et al., 2018). Animal studies using murine models have also shown a decreased activity of antioxidant enzymes following exposure to $PM_{2.5}$, leading to imbalances in reactive oxygen species and, consequently, to oxidative stress (Gangwar et al., 2020). Biomarkers of elevated oxidative stress are a well reported phenomena in patients with ASD (Pangrazzi et al., 2020). Repeated exposure to $PM_{2.5}$ has also been associated with mitochondrial damage (Sotty et al., 2020). $PM_{2.5}$ may also elicit epigenetic alterations: one study has shown that exposure to $PM_{2.5}$ induces DNA hypermethylation in

Table 4
Summary statistics regarding logistic regression stepwise models for first, second and third trimesters, full pregnancy and first year of life.

First Trimester					Second Trimester				
Variable	β	SE	p-value	OR (95% CI)	Variable	β	SE	p-value	OR (95% CI)
$PM_{2.5}$	**0.13**	**0.04**	**0.002**	**1.14 (1.05–1.23)**	$PM_{2.5}$	0.05	0.03	0.08	1.05 (0.99–1.12)
Sex	−1.03	0.62	0.10	0.36 (0.11–1.20)	Sex	−0.91	0.60	0.13	0.40 (0.13–1.29)
Maternal education	−0.66	0.41	0.10	0.51 (0.23–1.15)	Maternal education	−0.63	0.40	0.11	0.53 (0.24–1.16)
Third Trimester					**Full pregnancy**				
Variable	**β**	**SE**	**p-value**	**OR (95% CI)**	**Variable**	**β**	**SE**	**p-value**	**OR (95% CI)**
PM_{10}	**0.07**	**0.03**	**0.02**	**1.07 (1.01–1.14)**	**$PM_{2.5}$**	**0.07**	**0.03**	**0.04**	**1.07 (1.00–1.15)**
NO_2	−0.09	0.05	0.06	0.91 (0.83–1.00)	Sex	−0.97	0.60	0.10	0.38 (0.12–1.22)
Maternal education	−0.60	0.39	0.13	0.55 (0.26–1.18)	Maternal education	−0.64	0.40	0.11	0.53 (0.24–1.15)
First year of life									
Variable	**B**	**SE**	**p-value**	**OR (95% CI)**					
PM_{10}	0.04	0.03	0.16	1.04 (0.98–1.10)					
Sex	−0.99	0.60	0.10	0.37 (0.12–1.20)					
Maternal education	−0.67	0.40	0.09	0.51 (0.24–1.12)					

β – regression coefficient, SE – standard error, OR – Odds ratio, 95% CI – 95% Confidence interval.

promoter regions of ASD candidate genes, including *AUTS2, BDNF, GABRB3, GRIN1, MECP2, NLGN3, NRXN1, RELN, SHANK3* and *SLC6A4* leading to alterations in their mRNA and protein expression in human neurons (Wei et al., 2016). In fact, $PM_{2.5}$ exposure has been shown to shape global and gene-specific DNA methylation profiles throughout the lifespan, with marked effects during key life stages such as prenatal development and infancy (Ferrari et al., 2019).

When compared to $PM_{2.5}$, few studies are available on health risks associated with PM_{10} exposure (Yau et al., 2021. A recent study with murine models showed that PM_{10} induces neurotoxicity by promoting the death of dopaminergic neurons (Choi et al., 2022). PM_{10} exposure is also reported to induce epigenetic alterations (Ferrari et al., 2019), with a recent publication showing that prenatal exposure to PM_{10} was associated with altered methylation patterns and telomere length in human cord blood samples (Isaevska et al., 2022). Finer components of PM_{10}, including black carbon and metals, are able to cross the placenta (Giovannini et al., 2018; Bové et al., 2019), which justifies its neurodevelopmental outcomes and may underline an association with a higher clinical severity of ASD.

Our results suggest a time period specificity for the effects of $PM_{2.5}$ (first trimester and full pregnancy) and PM_{10} (third trimester). Early pregnancy is a period of heightened vulnerability to environmental toxins, and it has been suggested that $PM_{2.5}$ exposure during the first trimester promotes mitochondrial DNA damage at the placenta, leading to oxidative stress, and interfering with the barrier function of this structure (Grevendonk et al., 2016; Tapia et al., 2020). Maternal exposure to PM_{10} during late pregnancy leads to altered blood viscosity and clotting, resulting in a reduced flow of oxygen and nutrients between mother and child through the placenta (Chen et al., 2021). However, more studies are needed to experimentally assess neuropathological events caused by specific time period exposures.

Previous studies addressing an impact of exposure to air pollution during early life in ASD severity are scarce. An association between higher prenatal and first year NO_2 exposure with poorer performances on Mullen Scales of Early Learning and VABS scores, which measure cognitive and adaptive behavior, has been reported in a sample of 327 children with ASD (Kerin et al., 2018). Postnatal exposure to air pollutants has also been reported to modulate ASD severity, as observed by one study showing that high short-term exposure to $PM_{2.5}$, NO_2 and O_3 increased the risk of hospital admissions, which were used as a proxy for symptom aggravation, in Korean children with ASD aged 5–14 years old (Kim et al., 2022).

Although $PM_{2.5}$ and PM_{10} were the significant variables in the stepwise models, our results indicate that being female and a lower maternal education are also associated with a higher severity. Low maternal education is a strong predictor of lower IQ in the offspring (Camargo-Figuera et al., 2014; Beck et al., 2022), and our results show a not significant higher percentage of mothers with lower education in the higher (24.5%) versus milder (16.7%) severity groups. We also observed a non-significant trend of more females in the group with milder severity, which is unexpected since the male bias in ASD diagnoses tends to be less pronounced in groups with severe ID (Frazier et al., 2014). However, there is evidence for a differential response of male and female brains to air pollution, with males being more vulnerable to the adverse effects of in-utero exposure to air pollutants (Yi et al., 2022). A Mexican study further provides evidence for an interaction between air pollutant exposure and genetic background in neurodevelopmental disorders, by showing that in a population of children chronically-exposed to high concentrations of $PM_{2.5}$, young girls with Apolipoprotein E (APOE) 4 heterozygous allele presented increased odds of having low IQ scores (Calderon-Garcidueñas et al., 2016). Thus, integrating genetic data could result in models that best reflect the actual heterogeneous character of this disorder. Overall, our results suggest that, besides early-life exposure, sex and maternal education have a minor contribution to ASD severity in this population. Additionally, the inclusion of genetic susceptibility data may add and additional layer of complexity to these models.

Other clinical variables, including gestational age, birth weight and parental ages at birth, previously associated with ASD risk, did not impact on clinical severity. Epidemiologic studies that examine associations between these variables and ASD severity are scarce and present conflicting results. Regarding gestational age, a study has reported higher clinical severity in subjects with ASD from preterm births (Movsas and Paneth, 2012). However, these results were not replicated by a more recent study (Martini et al., 2022). Advanced paternal age, but not maternal age, at birth has been associated with higher clinical severity of ASD as measured by parental report, by one study (Rieske and Matson, 2020), a result not observed by others (Itzchak et al., 2011). Finally, one study found no association between very low birth weight and ASD severity (Itzchak et al., 2011). It is possible that these variables increase the risk of ASD, but have a more modest effect on modulating its severity that would require larger population studies to be detected (Martini et al., 2022).

The present study provides evidence that increased early-life exposure to PM, during critical neurodevelopmental windows, is not only a risk factor for ASD, but it is also associated with a higher clinical severity. This is in line with the notion that clinical stratification is fundamental to identify risk factors specific to clinical subgroups (Frye et al., 2019). Our study uses air pollution monitoring data to estimate maternal exposure during pregnancy, but does not directly measures the fetus exposure, identify which PM components drive the effect or examine the biological mechanisms by which the maternal exposure

may impact the brain development of the offspring. Future studies need to address these issues, eventually using biomarkers of exposure to PM, such as oxidative stress and inflammatory biomarkers (Desai et al., 2017; Friedman et al., 2021), as well as explore the biological mechanisms by which PM can cause neurodevelopmental impairments and modulate ASD clinical severity. Such studies could leverage brain organoids as a model system, which have already been used to test for neurotoxicity of PM (Bilinovich et al., 2020). Our results again highlight the importance of air quality for a healthy neurodevelopment and for the risk of severe neurodevelopmental disorders, raising questions that need to be addressed by public health policies.

Credit author statement

JXS, PS, CR and AMV developed the concept for this work. JXS and PS carried out data curation, methodological development, and formal analysis, with strong support from CR, CF, HM and LS, and with supervision from AN and AMV. CC, AO, FD and GO performed clinical evaluations. GO and AMV provided resources, including funding. AN and AMV performed the overall supervision and coordination. JXS wrote the initial draft, revised and edited by AMV and reviewed by PS, CR, CF, CC, AO, FD, GO, LS and AN. All authors contributed to the article and approved the submitted version.

Funding

This work was supported by *Fundação para a Ciência e a Tecnologia* (FCT), through funding to the project "Gene-environment interactions in Autism Spectrum Disorder" (Grant PTDC/MED-OUT/28937/2017) and to Research Center Grants UIDB/04046/2020 and UIDP/04046/2020 (to BioISI) and UIDB/00006/2020 (to Centro de Estatística e Aplicações da Universidade de Lisboa). João Xavier Santos is a fellow of the BioSys PhD Program and an awardee of a scholarship funded by FCT with reference PD/BD/114,386/2016.

Ethics statement

Informed consent to participate in the study was obtained for all subjects by their parents/legal guardian. Ethical approval was obtained by Comissão de Ética para a Saúde from Instituto Nacional de Saúde Doutor Ricardo Jorge, I.P. (INSA, I.P.) and by Comissão de Ética from Centro Hospitalar e Universitário de Coimbra (CE-CHUC).

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.

Acknowledgements

We acknowledge the Portuguese Environment Agency for the availability of air pollution data collected through the Portuguese air quality monitoring network. We are grateful to the families recruited through the Gene-Environment Interactions in Autism Spectrum Disorder (GEnvIA) project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2023.115795.

References

American Psychiatric Association, 2013. Diagnostic and Statistical Manual of Mental Disorders, fifth ed. American Psychiatric Association, Washington DC. https://doi.org/10.1176/appi.books.9780890425596.

Anderson, J.O., Thundiyil, J.G., Stolbach, A., 2012. Clearing the air: a review of the effects of particulate matter air pollution on human health. J. Med. Toxicol. 8 (2), 166–175. https://doi.org/10.1007/s13181-011-0203-1.

Bai, D., Yip, B.H.K., Windham, G.C., Sourander, A., Francis, R., Yoffe, R., et al., 2019. Association of genetic and environmental factors with autism in a 5-country cohort. JAMA Psychiatr. 76 (10), 1035–1043. https://doi.org/10.1001/jamapsychiatry.2019.1411.

Beck, I.H., Bilenberg, N., Davidsen, K.A., Rasmussen, A.A., Boye, H., Jensen, T.K., 2022. Prenatal and early childhood predictors of intelligence quotient (IQ) in 7-year-old Danish children from the Odense Child Cohort. Scand. J. Publ. Health. https://doi.org/10.1177/14034948221077463.

Becerra, T.A., Wilhelm, M., Olsen, J., Cockburn, M., Ritz, B., 2013. Ambient air pollution and autism in los angeles county, California. Environ. Health Perspect. 121 (3), 380–386. https://doi.org/10.1289/ehp.1205827.

Bilinovich, S.M., Uhl, K.L., Lewis, K., Soehnlen, X., Williams, M., Vogt, D., et al., 2020. Integrated RNA sequencing reveals epigenetic impacts of diesel particulate matter exposure in human cerebral organoids. Dev. Neurosci. 42 (5–6), 195–207. https://doi.org/10.1159/000513536.

Bové, H., Bongaerts, E., Slenders, E., Bijnens, E.M., Saenen, N.D., Gyselaers, W., Nawrot, T.S., 2019. Ambient black carbon particles reach the fetal side of human placenta. Nat. Commun. 10 (1), 1–7. https://doi.org/10.1038/s41467-019-11654-3.

Calderón-Garcidueñas, L., Jewells, V., Galaz-Montoya, C., van Zundert, B., Pérez-Calatayud, A., Ascencio-Ferrel, E., D'Angiulli, A., 2016. Interactive and additive influences of Gender, BMI and Apolipoprotein 4 on cognition in children chronically exposed to high concentrations of PM2. 5 and ozone. APOE 4 females are at highest risk in Mexico City. Environ. Res. 150, 411–422. https://doi.org/10.1016/j.envres.2016.06.026.

Camargo-Figuera, F.A., Barros, A.J., Santos, I.S., Matijasevich, A., Barros, F.C., 2014. Early life determinants of low IQ at age 6 in children from the 2004 Pelotas Birth Cohort: a predictive approach. BMC Pediatr. 14 (1), 1–12. https://doi.org/10.1186/s12887-014-0308-1.

Chen, X., Guo, J., Huang, Y., Liu, S., Huang, Y., Zhang, Z., Ding, W., 2020. Urban airborne PM2. 5-activated microglia mediate neurotoxicity through glutaminase-containing extracellular vesicles in olfactory bulb. Environ. Pollut. 264, 114716 https://doi.org/10.1016/j.envpol.2020.114716.

Chen, Y., Hodgson, S., Gulliver, J., Granell, R., Henderson, A.J., Cai, Y., et al., 2021. Trimester effects of source-specific PM10 on birth weight outcomes in the avon longitudinal study of parents and children (ALSPAC). Environ. Health 7 (1), 20. https://doi.org/10.1186/s12940-020-00684-w, 4.

Cheroni, C., Caporale, N., Testa, G., 2020. Autism spectrum disorder at the crossroad between genes and environment: contributions, convergences, and interactions in ASD developmental pathophysiology. Mol. Autism. 11 (1), 1–18. https://doi.org/10.1186/s13229-020-00370-1.

Choi, D., Lee, G., Kim, H.K., Bae, H., 2022. Particulate Matter Exacerbates the Death of Dopaminergic Neurons in Parkinson's Disease through an Inflammatory Response. Int. J. Mol. Sci. 10 23 (12), 6487. https://doi.org/10.3390/ijms23126487.

Costa, L.G., Cole, T.B., Dao, K., Chang, Y.C., Coburn, J., Garrick, J.M., 2020. Effects of air pollution on the nervous system and its possible role in neurodevelopmental and neurodegenerative disorders. Pharmacol. Ther. 210, 107523 https://doi.org/10.1016/j.pharmthera.2020.107523.

Cutland, C.L., Lackritz, E.M., Mallett-Moore, T., Bardají, A., Chandrasekaran, R., Lahariya, C. al, 2017. Low birth weight: case definition and guidelines for data collection, analysis, and presentation of maternal immunization safety data. Vaccine 35 (48), 6492. https://doi.org/10.1016/j.vaccine.2017.01.049.

de la Torre-Ubieta, L., Won, H., Stein, J.L., Geschwind, D.H., 2016. Advancing the understanding of autism disease mechanisms through genetics. Nat. Med. 22 (4), 345–361. https://doi.org/10.1038/nm.4071.

Desai, G., Chu, L., Guo, Y., Myneni, A.A., Mu, L., 2017. Biomarkers used in studying air pollution exposure during pregnancy and perinatal outcomes: a review. Biomarkers 22 (6), 489–501. https://doi.org/10.1080/1354750X.2017.1339294.

Eftekharian, M.M., Ghafouri-Fard, S., Noroozi, R., Omrani, M.D., Arsang-Jang, S., Ganji, M., et al., 2018. Cytokine profile in autistic patients. Cytokine 108, 120–126. https://doi.org/10.1016/j.cyto.2018.03.034.

Familari, M., Nääv, Å., Erlandsson, L., de Iongh, R.U., Isaxon, C., Strandberg, B., Malmqvist, E., 2019. Exposure of trophoblast cells to fine particulate matter air pollution leads to growth inhibition, inflammation and ER stress. PLoS One 14 (7), e0218799. https://doi.org/10.1371/journal.pone.0218799.

Ferrari, L., Carugno, M., Bollati, V., 2019. Particulate matter exposure shapes DNA methylation through the lifespan. Clin. Epigenet. 11 (1), 1–14. https://doi.org/10.1186/s13148-019-0726-x.

Fleury, V., Himsl, R., Joost, S., Nicastro, N., Bereau, M., Guessous, I., Burkhard, P.R., 2021. Geospatial analysis of individual-based Parkinson's disease data supports a link with air pollution: a case-control study. Parkinsonism Relat. Disord. 83, 41–48. https://doi.org/10.1016/j.parkreldis.2020.12.013.

Fossé, N.A., Hoorn, M., Lith, J., Cessie, S., Lashley, E., 2020. Advanced paternal age is associated with an increased risk of spontaneous miscarriage: a systematic review and meta-analysis. Hum. Reprod. Update 26 (5), 650–669. https://doi.org/10.1093/humupd/dmaa010.

Frazier, W.E., Thompson, L., Youngstrom, E.A., Law, P., Hardan, A.Y., et al., 2014. A twin study of heritable and shared environmental contributions to autism. J. Autism Dev. Disord. 44 (8), 2013–2025. https://doi.org/10.1007/s10803-014-2081-2.

Friedman, C., Dabelea, D., Thomas, D.S., Peel, J.L., Adgate, J.L., Magzamen, S., et al., 2021. Exposure to ambient air pollution during pregnancy and inflammatory biomarkers in maternal and umbilical cord blood: the Healthy Start study. Environ. Res. 197, 111165 https://doi.org/10.1016/j.envres.2021.111165.

Frye, R.E., Vassall, S., Kaur, G., Lewis, C., Karim, M., Rossignol, D., 2019. Emerging biomarkers in autism spectrum disorder: a systematic review. Ann. Transl. Med. 7 (23) https://doi.org/10.21037/atm.2019.11.53.

Gaio, V., Roquette, R., Monteiro, A., Ferreira, J., Lopes, D., Dias, C.M., Nunes, B., 2022. PM10 exposure interacts with abdominal obesity to increase blood triglycerides: a cross-sectional linkage study. Eur. J. Public Health. 32 (2), 281–288. https://doi.org/10.1093/eurpub/ckab190.

Gangwar, R.S., Bevan, G.H., Palanivel, R., Das, L., Rajagopalan, S., 2020. Oxidative stress pathways of air pollution mediated toxicity: recent insights. Redox Biol. 34, 101545 https://doi.org/10.1016/j.redox.2020.101545.

Geng, R., Fang, S., Li, G., 2019. The association between particulate matter 2.5 exposure and children with autism spectrum disorder. Int. J. Dev. Neurosci. 75, 59–63. https://doi.org/10.1016/j.ijdevneu.2019.05.003.

Genovese, A., Butler, M.G., 2020. Clinical assessment, genetics, and treatment approaches in autism spectrum disorder (ASD). Int. J. Mol. Sci. 21 (13), 4726. https://doi.org/10.3390/ijms21134726.

Ghazi, T., Naidoo, P., Naidoo, R.J., Chuturgoon, A.A., 2021. Prenatal Air Pollution Exposure and Placental DNA Methylation Changes: Implications on Fetal Development and Future Disease Susceptibility. Cells 10 (11), 3025. https://doi.org/10.3390/cells10113025.

Giovannini, N., Schwartz, L., Cipriani, S., Parazzini, F., Baini, I., Signorelli, V., Cetin, I., 2018. Particulate matter (PM10) exposure, birth and fetal-placental weight and umbilical arterial pH: results from a prospective study. J. Matern. Fetal Neonat. Med. 31 (5), 651–655. https://doi.org/10.1080/14767058.2017.1293032.

Glick, I., Kadish, E., Rottenstreich, M., 2021. Management of pregnancy in women of advanced maternal age: improving outcomes for mother and baby. Int. J. Womens Health 13, 751. https://doi.org/10.2147/IJWH.S283216.

Gotham, K., Pickles, A., Lord, C., 2009. Standardizing ADOS scores for a measure of severity in autism spectrum disorders. J. Autism Dev. Disord. 39 (5), 693–705. https://doi.org/10.1007/s10803-008-0674-3.

Grevendonk, L., Janssen, B.G., Vanpoucke, C., Lefebvre, W., Hoxha, M., Bollati, V., et al., 2016. Mitochondrial oxidative DNA damage and exposure to particulate air pollution in mother-newborn pairs. Environ. Health. 20, 15. https://doi.org/10.1186/s12940-016-0095-2, 10.

Guerreiro, C.B., Foltescu, V., De Leeuw, F., 2014. Air quality status and trends in Europe. Atmos. Environ. 98, 376–384. https://doi.org/10.1016/j.atmosenv.2014.09.017.

Isaevska, E., Fiano, V., Asta, F., Stafoggia, M., Moirano, G., Popovic, M., et al., 2022. Prenatal exposure to PM_{10} and changes in DNA methylation and telomere length in cord blood. Environ. Res. 209, 112717 https://doi.org/10.1016/j.envres.2022.112717.

Itzchak, E.B., Lahat, E., Zachor, D.A., 2011. Advanced parental ages and low birth weight in autism spectrum disorders—rates and effect on functioning. Res. Dev. Disabil. 32 (5), 1776–1781. https://doi.org/10.1016/j.ridd.2011.03.004.

Janeczko, D., Hołowczuk, M., Orzeł, A., Klatka, B., Semczuk, A., 2020. Paternal age is affected by genetic abnormalities, perinatal complications and mental health of the offspring. Biomed. Rep. 12 (3), 83–88. https://doi.org/10.3892/br.2019.1266.

Jung, C.R., Lin, Y.T., Hwang, B.F., 2013. Air pollution and newly diagnostic autism spectrum disorders: a population-based cohort study in Taiwan. PLoS One 8 (9), e75510. https://doi.org/10.1371/journal.pone.0075510.

Kalkbrenner, A.E., Windham, G.C., Serre, M.L., Akita, Y., Wang, X., Hoffman, K., et al., 2015. Particulate matter exposure, prenatal and postnatal windows of susceptibility, and autism spectrum disorders. Epidemiology 26 (1), 30–42. https://doi.org/10.1097/EDE.0000000000000173.

Kaufman, J.A., Wright, J.M., Rice, G., Connolly, N., Bowers, K., Anixt, J., 2019. Ambient ozone and fine particulate matter exposures and autism spectrum disorder in metropolitan Cincinnati, Ohio. Environ. Res. 171, 218–227. https://doi.org/10.1016/j.envres.2019.01.013.

Krakowiak, P., Goines, P.E., Tancredi, D.J., Ashwood, P., Hansen, R.L., Hertz-Picciotto, I., Van de Water, J., 2017. Neonatal cytokine profiles associated with autism spectrum disorder. Biol. Psychiatry. 81 (5), 442–451. https://doi.org/10.1016/j.biopsych.2015.08.007.

Kerin, T., Volk, H., Li, W., Lurmann, F., Eckel, S., McConnell, R., et al., 2018. Association between air pollution exposure, cognitive and adaptive function, and ASD severity among children with autism spectrum disorder. J. Autism Dev. Disord. 48 (1), 137–150. https://doi.org/10.1007/s10803-017-3304-0.

Kim, D., Volk, H., Girirajan, S., Pendergrass, S., Hall, M.A., Verma, S.S., et al., 2017. The joint effect of air pollution exposure and copy number variation on risk for autism. Autism Res. 10 (9), 1470–1480. https://doi.org/10.1002/aur.1799.

Kim, H., Kim, W.H., Kim, Y.Y., Park, H.Y., 2020. Air pollution and central nervous system disease: a review of the impact of fine particulate matter on neurological disorders. Front. Public Health 8, 575330. https://doi.org/10.3389/fpubh.2020.575330.

Kim, K.N., Sohn, J.H., Cho, S.J., Seo, H.Y., Kim, S., Hung, Y.C., 2022. Effects of short-term exposure to air pollution on hospital admissions for autism spectrum disorder in Korean school-aged children: a nationwide time-series study. BMJ Open. https://doi.org/10.1136/bmjopen-2021-058286.

Lee, B.K., Gross, R., Francis, R.W., Karlsson, H., Schendel, D.E., Sourander, A., et al., 2019. Birth seasonality and risk of autism spectrum disorder. Eur. J. Epidemiol. 34 (8), 785–792. https://doi.org/10.1007/s10654-019-00506-5.

Lord, C., Rutter, M., Le Couteur, A., 1994. Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J. Autism Dev. Disord. 24 (5), 659–685. https://doi.org/10.1007/BF02172145.

Lord, C., Risi, S., Lambrecht, L., Cook, E.H., Leventhal, B.L., DiLavore, P.C., et al., 2000. The Autism Diagnostic Observation Schedule—generic: A standard measure of social and communication deficits associated with the spectrum of autism. J. Autism Dev. Disord. 30 (3), 205–223. https://doi.org/10.1023/A:1005592401947.

Lord, C., Brugha, T.S., Charman, T., Cusack, J., Dumas, G., Frazier, T., et al., 2020. Autism spectrum disorder. Nat. Rev. Dis. Primers. 6 (1), 1–23. https://doi.org/10.1038/s41572-019-0138-4.

Liu, F., Huang, Y., Zhang, F., Chen, Q., Wu, B., Rui, W., et al., 2015. Macrophages treated with particulate matter PM 2.5 induce selective neurotoxicity through glutaminase-mediated glutamate generation. J. Neurochem. 134 (2), 315–326. https://doi.org/10.1111/jnc.13135.

Lyall, K., Schmidt, R.J., Hertz-Picciotto, I., 2014. Maternal lifestyle and environmental risk factors for autism spectrum disorders. Int. J. Epidemiol. 43 (2), 443–464. https://doi.org/10.1093/ije/dyt282.

McPartland, J.C., Bernier, R.A., Jeste, S.S., Dawson, G., Nelson, C.A., Chawarska, K., et al., 2020. The autism biomarkers consortium for clinical trials (ABC-CT): scientific context, study design, and progress toward biomarker qualification. Front. Integr. Neurosci. 14, 16. https://doi.org/10.3389/fnint.2020.00016.

Manoli, D.S., State, M.W., 2021. Autism spectrum disorder genetics and the search for pathological mechanisms. Am. J. Psychiatry. 178 (1), 30–38. https://doi.org/10.1176/appi.ajp.2020.20111608.

Martini, M.I., Merkelbach, I., Begeer, S., 2022. Gestational age in children and adolescents: prevalence and effects on autism phenotype. J. Autism Dev. Disord. https://doi.org/10.1007/s10803-022-05466-6.

Masi, A., Glozier, N., Dale, R., Guastella, A.J., 2017. The immune system, cytokines, and biomarkers in autism spectrum disorder. Neurosci. Bull. 33 (2), 194–204. https://doi.org/10.1007/s12264-017-0103-8.

Meter, K.C., Christiansen, L.E., Delwiche, L.D., Azari, R., Carpenter, T.E., Hertz-Picciotto, I., 2010. Geographic distribution of autism in California: a retrospective birth cohort analysis. Autism Res. 3 (1), 19–29. https://doi.org/10.1002/aur.110.

Ministério do Ambiente e Ordenamento do Território, Direção Geral do Ambiente, 2001. Delimitação de zonas e aglomerações para avaliação da qualidade do ar em Portugal (in Portuguese).

Modabbernia, A., Velthorst, E., Reichenberg, A., 2017. Environmental risk factors for autism: an evidence-based review of systematic reviews and meta-analyses. Mol. Autism. 8 (1), 13. https://doi.org/10.1186/s13229-017-0121-4.

Moore, T., Arefadib, N., Deery, A., West, S., Keyes, M., 2017. The First Thousand Days: an Evidence Paper-Summary.

Monteiro, A., Miranda, A.I., Borrego, C., Vautard, R., 2007. Air quality assessment for Portugal. Sci. Total Environ. 373 (1), 22–31. https://doi.org/10.1016/j.scitotenv.2006.10.014.

Monteiro, A., Ferreira, J., Ribeiro, I., Fernandes, A.P., Martins, H., Gama, C., et al., 2015. Air quality over Portugal in 2020. Atmos. Poll. Res. 6 (5), 788–796. https://doi.org/10.5094/APR.2015.087.

Movsas, T.Z., Paneth, N., 2012. The effect of gestational age on symptom severity in children with Autism Spectrum Disorder. J. Autism Dev. Disord. 42, 2431–2439. https://doi.org/10.1007/s10803-012-1501-4.

Oliveira, G., Ataíde, A., Marques, C., Miguel, T.S., Coutinho, A.M., Mota-Vieira, L., et al., 2007. Epidemiology of autism spectrum disorder in Portugal: prevalence, clinical characterization, and medical conditions. Dev. Med. Child Neurol. 49 (10), 726–733. https://doi.org/10.1111/j.1469-8749.2007.00726.x.

Ongono, S. J., Michelon, C., Béranger, R., Cadot, E., Simoncic, V., Loubersac, J. et al., 2022, Association between residential proximity to agricultural crops and adaptive behaviors in children with autism spectrum disorder from the French ELENA cohort. J. Psychiat. Res. 145:197-204. doi: 10.1016/j.jpsychires.2021.12.007.

Pangrazzi, L., Balasco, L., Bozzi, Y., 2020. Oxidative stress and immune system dysfunction in autism spectrum disorders. Int. J. Mol. Sci. 21 (9), 3293. https://doi.org/10.3390/ijms21093293.

Pettersson, E., Lichtenstein, P., Larsson, H., Song, J., Deficit, A., Agrawal, A., Polderman, T.J.C., 2019. Genetic influences on eight psychiatric disorders based on family data of 4 408 646 full and half-siblings, and genetic data of 333 748 cases and controls. Psychol. Med. 49 (7), 1166–1173. https://doi.org/10.1017/S0033291718002945.

Pope III, C.A., Bhatnagar, A., McCracken, J.P., Abplanalp, W., Conklin, D.J., et al., 2016. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Cir. Res. 119 (11), 1204–1214. https://doi.org/10.1161/CIRCRESAHA.116.309279.

Rahman, M.M., Shu, Y.H., Chow, T., Lurmann, F.W., Yu, X., Martinez, M.P., et al., 2022. Prenatal exposure to air pollution and autism spectrum disorder: sensitive windows of exposure and sex differences. Environ. Health Perspect. 130 (1), 17008 https://doi.org/10.1289/EHP9509.

Raz, R., Levine, H., Pinto, O., Broday, D.M., Weisskopf, M.G., 2018. Traffic-related air pollution and autism spectrum disorder: a population-based nested case-control study in Israel. Am. J. Epidemiol. 187 (4), 717–725. https://doi.org/10.1093/aje/kwx294.

Raz, R., Roberts, A.L., Lyall, K., Hart, J.E., Just, A.C., Laden, F., Weisskopf, M.G., 2015. Autism spectrum disorder and particulate matter air pollution before, during, and after pregnancy: a nested case–control analysis within the Nurses' Health Study II cohort. Environ. Health Perspect. 123 (3), 264–270. https://doi.org/10.1289/ehp.1408133.

Rossignol, D.A., Genuis, S.J., Frye, R.E., 2014. Environmental toxicants and autism spectrum disorders: a systematic review. Transl. Psychiatry 4 (2). https://doi.org/10.1038/tp.2014.4 e360-e360.

Rieske, R.D., Matson, J.L., 2020. Parental age at conception and the relationship with severity of autism symptoms. Dev. Neurorehabil. 23 (5), 265–270. https://doi.org/10.1080/17518423.2019.1645222.

Rylaarsdam, L., Guemez-Gamboa, A., 2019. Genetic causes and modifiers of autism spectrum disorder. Front. Cell. Neurosci. 385 https://doi.org/10.3389/fncel.2019.00385.

Sandin, S., Lichtenstein, P., Kuja-Halkola, R., Hultman, C., Larsson, H., Reichenberg, A., 2017. The heritability of autism spectrum disorder. JAMA 318 (12), 1182–1184. https://doi.org/10.1001/jama.2017.12141.

Santos, J.X., Rasga, C., Vicente, A.M., 2021. Exposure to xenobiotics and gene-environment interactions in autism spectrum disorder: a systematic review. Autism Spectrum Disorder-Profile, Heterogeneity, Neurobiology and Intervention 133. https://doi.org/10.5772/intechopen.95758.

Santos, J.X., Rasga, C., Marques, A.R., Martiniano, H., Asif, M., Vilela, J., et al., 2022. A role for gene-environment interactions in autism spectrum disorder is supported by variants in genes regulating the effects of exposure to xenobiotics. Front. Neurosci. 16 https://doi.org/10.3389/fnins.2022.862315.

Sicard, P., Agathokleous, E., De Marco, A., Paoletti, E., Calatayud, V., 2021. Urban population exposure to air pollution in Europe over the last decades. Environ. Sci. Eur. 33 (1), 1–12. https://doi.org/10.1186/s12302-020-00450-2.

Sotty, J., Kluza, J., De Sousa, C., Tardivel, M., Anthérieu, S., Alleman, L.Y., et al., 2020. Mitochondrial alterations triggered by repeated exposure to fine (PM2. 5-0.18) and quasi-ultrafine (PM0. 18) fractions of ambient particulate matter. Environ. Int. 142, 105830 https://doi.org/10.1016/j.envint.2020.105830.

Sparrow, S.S., Cicchetti, D.V., Saulnier, C.A., 2016 (Vineland-3). *Vineland adaptive behavior scales*, Third edition. NCS Pearson, Bloomington.

Talbott, E.O., Arena, V.C., Rager, J.R., Clougherty, J.E., Michanowicz, D.R., Sharma, R. K., Stacy, S.L., 2015. Fine particulate matter and the risk of autism spectrum disorder. Environ. Res. 140, 414–420. https://doi.org/10.1016/j.envres.2015.04.021.

Tapia, V.L., Vasquez, B.V., Vu, B., Liu, Y., Steenland, K., Gonzales, G.F., 2020. Association between maternal exposure to particulate matter (PM2.5) and adverse pregnancy outcomes in Lima, Peru. J. Expo. Sci. Environ. Epidemiol. 30 (4), 689–697. https://doi.org/10.1038/s41370-020-0223-5.

Van Heijst, B.F., Geurts, H.M., 2015. Quality of life in autism across the lifespan: a meta-analysis. Autism 19 (2), 158–167. https://doi.org/10.1177/1362361313517053.

van't Hof, M., Tisseur, C., van Berckelear-Onnes, I., van Nieuwenhuyzen, A., Daniels, A. M., Deen, M., et al., 2021. Age at autism spectrum disorder diagnosis: a systematic review and meta-analysis from 2012 to 2019. Autism 25 (4), 862–873. https://doi.org/10.1177/1362361320971107.

Volk, H.E., Lurmann, F., Penfold, B., Hertz-Picciotto, I., McConnell, R., 2013. Traffic-related air pollution, particulate matter, and autism. JAMA Psychiatr. 70 (1), 71–77. https://doi.org/10.1001/jamapsychiatry.2013.266.

Volk, H.E., Kerin, T., Lurmann, F., Hertz-Picciotto, I., McConnell, R., et al., 2014. Interaction of the MET receptor tyrosine kinase gene and air pollution exposure in autism spectrum disorder. Epidemiology 25 (1), 44. https://doi.org/10.1097/EDE.0000000000000030.

Volk, H.E., Perera, F., Braun, J.M., Kingsley, S.L., Gray, K., Buckley, J., et al., 2021. Prenatal air pollution exposure and neurodevelopment: a review and blueprint for a harmonized approach within ECHO. Environ. Res. 196 https://doi.org/10.1016/j.envres.2020.110320.

Wei, H., Liang, F., Meng, G., Nie, Z., Zhou, R., Cheng, W., et al., 2016. Redox/methylation mediated abnormal DNA methylation as regulators of ambient fine particulate matter-induced neurodevelopment related impairment in human neuronal cells. Sci. Rep. 6 (1), 1–13. https://doi.org/10.1038/srep33402.

Werder, E.J., Engel, L.S., Lawrence, K.G., Sandler, D.P., 2021. Ambient particulate matter, ozone, and neurologic symptoms in US Gulf states adults. Environ. Epidemiol. 5 (4) https://doi.org/10.1097/EE9.0000000000000160.

World Health Organization, 2005. Air Quality Guidelines, Global Update. WHO Regional Office for Europe, Copenhagen, Denmark.

World Health Organization, 2021. Air Pollution and Child Health: Prescribing Clean Air. WHO Regional Office for Europe, Copenhagen, Denmark.

Wu, S., Wu, F., Ding, Y., Hou, J., Bi, J., Zhang, Z., 2017. Advanced parental age and autism risk in children: a systematic review and meta-analysis. Acta Psychiatr. Scand. 135 (1), 29–41. https://doi.org/10.1111/acps.12666.

Yau, S.Y., Lu, H.X., Lee, T.M., Guo, H., Chan, C.C., 2021. PM2. 5 as a potential risk factor for autism spectrum disorder: its possible link to neuroinflammation, oxidative stress and changes in gene expression. Neurosci. Biobehav. Rev. 128, 534–548. https://doi.org/10.1016/j.neubiorev.2021.06.043.

Yi, C., Wang, Q., Qu, Y., Niu, J., Oliver, B.G., Chen, H., 2022. In-utero exposure to air pollution and early-life neural development and cognition. Ecotoxicol. Environ. Saf. 238, 113589 https://doi.org/10.1016/j.ecoenv.2022.113589.

Zhang, Y., Wang, S., Zhu, J., Li, C., Zhang, T., Liu, H., Ye, L., 2018. Effect of atmospheric PM2. 5 on expression levels of NF-κB genes and inflammatory cytokines regulated by NF-κB in human macrophage. Inflammation 41 (3), 784–794. https://doi.org/10.1007/s10753-018-0732-8.

Zhu, N., Ji, X., Geng, X., Yue, H., Li, G., Sang, N., 2021. Maternal PM2. 5 exposure and abnormal placental nutrient transport. Ecotoxicol. Environ. Saf. 207 https://doi.org/10.1016/j.ecoenv.2020.111281.

Zou, W., Wang, X., Hong, W., He, F., Hu, J., Sheng, Q., Ran, P., 2020. PM2. 5 induces the expression of inflammatory cytokines via the Wnt5a/Ror2 pathway in human bronchial epithelial cells. Int. J. Chronic Obstr. Pulm. Dis. 15, 2653. https://doi.org/10.2147/COPD.S270762.