Socio-environmental exposures and neurodevelopmental disorders

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Al Cormac i al Fionn

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"The second goal of education is to form minds which can be critical, can verify, and not accept everything they are offered. The great danger today is of slogans, collective opinions, ready-made trends of thought. We have to be able to resist individually, to criticize, to distinguish between what is proven and what is not."

Jean Piaget, 1964

Duckworth, E. (1964). Piaget rediscovered. *The Arithmetic Teacher, 11*(7), 496-499.

Summary

Introduction

The influence of the environment, and its interplay with genetics, can account for up to half of the variance of neurodevelopmental disorders. In this thesis we reviewed previous literature on air pollution and neuropsychological development. We studied the trajectories of attention development in children. Finally, we explored any association between socio-environmental factors and warning signs of dyslexia and ADHD (attention deficit hyperactivity disorder) symptoms.

Methods

This thesis is part of the BREATHE (BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn) and the INMA (INfancia y Medio Ambiente) projects.

Results

(1) We encountered sufficient evidence for an association between outdoor air pollution and a negative impact on the neuropsychological development of children. (2) We detected an ongoing development of some attention processes in primary school children, differentiating patterns by gender and ADHD symptoms. (3) Daily levels of traffic-related ambient air pollution were associated with daily variations in attention processes in primary school children. (4) Prenatal, and at some extent postnatal, exposure to NO_2 at the residence address increased the risk of presenting signs of dyslexia in primary school children. (5) Within a total of 23 socio-environmental factors, only male gender, younger relative age, psychosocial adversity, smoking and prenatal exposure to alcohol associated with ADHD symptoms at preschool age using a novel method for handling correlated data.

Conclusions

Traffic-related air pollution, smoking, alcohol, gender, relative age and psychosocial adversity were identified as important determinants of neurodevelopmental disorders.

Resum

Introducció

La influència del medi ambient, i la seva interacció amb la genètica, pot explicar fins a la meitat de la variància dels trastorns del neurodesenvolupament. En aquesta tesi s'ha fet una revisió sobre la contaminació atmosfèrica i el desenvolupament neuropsicològic. S'han estudiat les trajectòries del desenvolupament de l'atenció en nens/es. Finalment, s'ha explorat l'associació entre factors socioambientals i indicadors d'alt risc de dislèxia i símptomes de TDAH (trastorn per dèficit d'atenció i hiperactivitat).

Mètodes

Aquesta tesi forma part dels projectes BREATHE (BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn) i INMA (INfància i Medi Ambient).

Resultats

(1) Hem trobat suficient evidència d'una associació entre la contaminació atmosfèrica i un impacte negatiu en el desenvolupament neuropsicològic dels nens/es. (2) Hem detectat trajectòries del desenvolupament de l'atenció en nens/es de primària, diferenciant patrons per gènere i símptomes de TDAH. (3) Els nivells diaris de contaminació atmosfèrica relacionada amb el trànsit es van associar amb variacions diàries en els processos atencionals en nens/es de primària. (4) L'exposició prenatal, i en certa mesura la postnatal, a la contaminació atmosfèrica relacionada amb el trànsit va augmentar el risc de presentar indicadors de dislèxia en els nens/es d'educació primària. (5) D'entre un total de 23 factors socio ambientals, només el gènere masculí, menys edat relativa (els nens/es més petits de la classe), l'adversitat psicosocial, el tabaquisme i l'exposició prenatal a l'alcohol es van associar amb símptomes de TDAH a l'edat preescolar utilitzant un mètode novedós per analitzar dades correlacionades.

Conclusions

La contaminació atmosfèrica relacionada amb el trànsit, el tabaquisme, l'alcohol, el gènere, l'edat relativa i l'adversitat psicosocial es van identificar com a determinants importants dels trastorns del neurodesenvolupament.

Preface

This thesis consists of a compilation of the scientific publications co-authored by the PhD candidate, supervised by Prof. Jordi Sunyer Deu, Dr. Joan Forns and Dr. Anna López Sala, according to the procedures of the Biomedicine PhD program of the Department of Experimental and Health Sciences of Universitat Pompeu Fabra. The book includes an abstract, a general introduction, the thesis' rationale and objectives, the methods, the research results (four original papers and a review article, three of them published), a global discussion and final conclusions.

The present thesis aims to improve the knowledge on cognitive development and the role of the environment (including physical and psychosocial factors) in neurodevelopmental disorders such as dyslexia and ADHD (attention deficit and hyperactivity disorder). Specifically, it contributed to: 1) update previous reviews on air pollution and neuropsychological development in children, 2) broaden knowledge about the development of attention processes in primary school children, 3) examine the acute effects of traffic-related air pollution on attention in primary school children, 4) the investigation of any association between pre- and postnatal exposure to traffic-related air pollution and warning signs of dyslexia in primary school children, 5) the understanding of pre- and postnatal exposures, including physical and psychosocial factors, associated with ADHD symptoms in preschool-aged children using a novel statistical method to deal with correlated data.

The publications are based on data from the BREATHE project, a 7-11 year-olds cohort in the city of Barcelona (Spain), and the INMA project, a population-based birth cohort composed by seven cohorts in different regions of Spain.

The work, which included conceptualization of the studies, statistical analysis, writing of the articles, and some field work, was performed by the PhD candidate at the Barcelona Institute for Global Health (ISGlobal) Campus Mar.

Contents

1. INTRODUCTION

1.1 First section: Development of the brain and its vulnerability

The development of the central nervous system (CNS) starts early in embryogenesis through the process called neurulation. In humans, neural tube formation is complete on approximately gestational day 28. From the first month of gestation, specific areas of the CNS begin to form with the neurogenesis, followed by a sequence of developmental processes including proliferation, migration, differentiation, synaptogenesis, apoptosis and myelination. The postnatal period is marked by increased cortical complexity, with both synaptogenesis and myelination continuing through adolescence and even adulthood (synaptic plasticity) (Rice & Barone, 2000) (**Figure 1**).

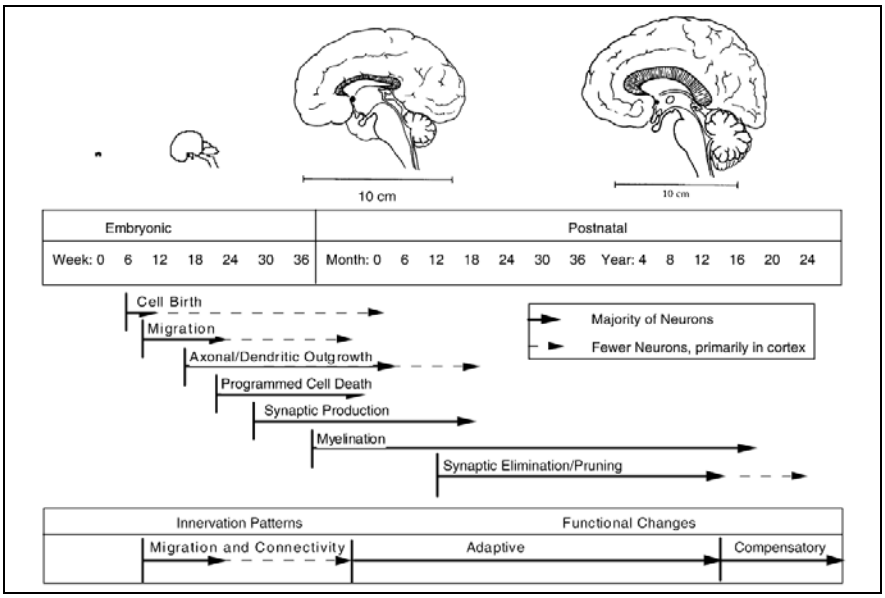

Figure 1. The stages of brain development (top) and different windows of vulnerability (bottom). Developmental processes occur in phases, setting the stage for potential periods of vulnerability. Insults early in life (bottom) will be assimilated into innervation patterns, whereas a later pre-pubertal insult will cause functional changes that are more adaptive. Reproduced from Andersen, 2003.

During postnatal life, pathways to the primary motor, somatosensory, visual and auditory cortical areas are the first to myelinate. At later developmental stages, myelin spreads towards

other cortical areas, being the frontal and parietal areas the latter to mature (Guillery, 2005) (**Figure 2**).

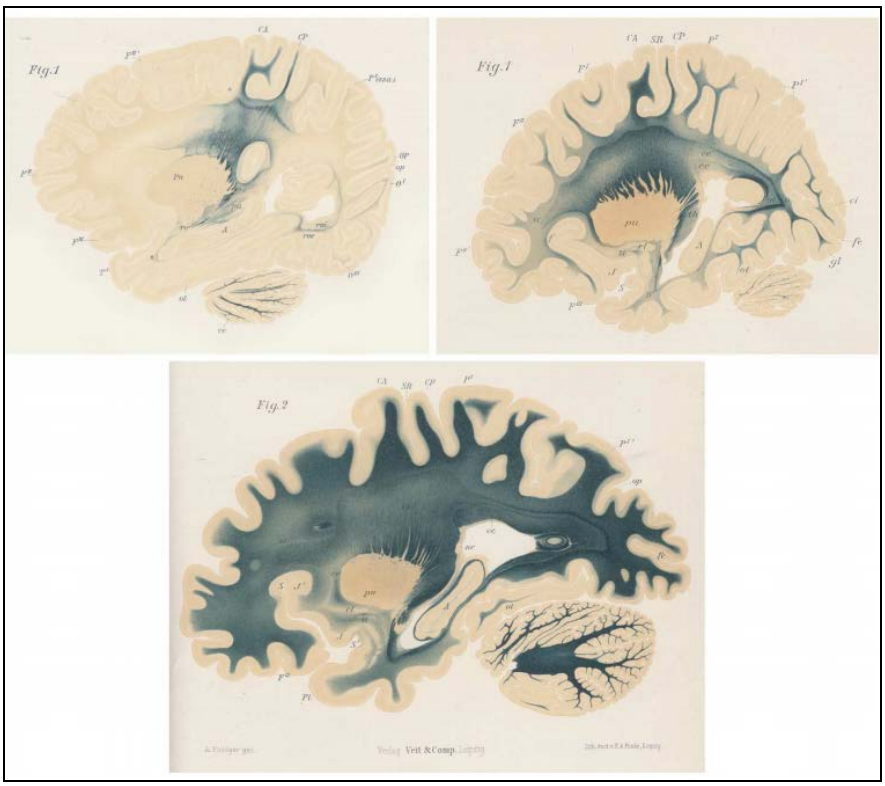

Figure 2. Development of myelin in the human brain. Parasagittal sections stained using the Weigert method show development of myelin in the human brain at birth (top left), at four months of age (top right) and in the adult (bottom). At birth, the white matter underlying the primary sensory and motor cortical areas shows myelination (dark blue), and some of the myelinated axons running towards the visual cortex are visible. The auditory cortex lies in more lateral sections (not shown) and also has myelinated white matter. At later developmental stages, myelin spreads towards other cortical areas. Note the late myelination of the frontal and parietal cortical areas. Reproduced from Guillery, 2005 [1].

Normal brain development includes apoptosis. There are two waves of pruning of synapses that are surplus or underused with the hypothesized goal to increase efficiency of synaptic transmission: the period immediately before birth and during the periadolescent period (Andersen, 2003).

These anatomical changes during brain maturation agree with regionally relevant milestones in cognitive and functional development. Motor and sensory brain areas mature first, followed

[1] Reproduced from Flechsig, P.E. (1920) *Anatomie des Menschlichen Gehirn und Ru¨ckenmarks, auf Myelogenetischer Grundlage*, G. Thieme.

by areas involved in spatial orientation, speech and language development, and attention (upper and lower parietal lobes). Later to mature are areas involved in executive function, attention and motor coordination (frontal lobes). The frontal pole, involved in taste and smell processing, and the occipital pole, containing the primary visual cortex, also mature early (Gogtay et al., 2004).

From the summary of the brain development processes, it is evident that the temporal window of vulnerability starts from day 1 in fetal life. Specifically, the vulnerability is dependent on two main exposure issues: whether an agent reaches the CNS and the maturational stage of exposure (Rice & Barone, 2000). The immature organism adapts by incorporating environmental information permanently into the mature structure and function. In contrast, the mature organism compensates to accommodate changes in the environment (**Figure 1**) (Andersen, 2003). The consequences of the timing of exposure to environmental agents and effects may be manifested later in life.

1.2 Second section: Neurodevelopmental disorders

The term *development* accurately describes the fact that neurodevelopmental disorders (ND) are probably the result of prenatal, and to a lesser extent early postnatal, brain maturation (Gilger & Kaplan, 2001). Individuals with ND have an atypically developed or functioning brain. Two categories of influences can affect the brain: the genes and the environment. The genetics of ND are complex due to their multigenic etiology and only candidate genes (neither necessary nor sufficient) with small risk for the disorders are identified (Gizer, Ficks, & Waldman, 2009). Furthermore, environmental influences and gene-environment interactions are likely to be involved in the development of complex disorders accounting for some quarter to a half of the variance (Plomin, Owen, & McGuffin, 1994). Comorbidity of ND is high due to shared etiological factors. For instance, across studies around 25-40% of children with either dyslexia or ADHD also meet criteria for the other disorder (Pennington, 2006).

a) Dyslexia

Dyslexia is one of the most common ND with a prevalence rate of around 7% (Peterson & Pennington, 2012) and it refers to a specific reading disability despite educational opportunity and adequate intelligence. The neural correlates of dyslexia across languages (white matter changes in posterior and anterior left hemisphere language networks) are well documented (Peterson & Pennington, 2012). There is evidence for its high heritability rates (up to 75%) although the influence of environmental factors, and their interplay with genetics, cannot be dismissed (Olson, 2006; Peterson & Pennington, 2012). Previous literature pointed out that family socioeconomic status (e.g., parents' education level, language and literacy environment) (Olson, 2006; Peterson & Pennington, 2012), and child health risks (e.g., gestational age) (Kovachy, Adams, Tamaresis, & Feldman, 2015) are the main environmental determinants of dyslexia. However, much remains to be learned about the role of the environment in its development.

b) ADHD

ADHD is another common ND, with an estimated prevalence ranging from 8 to 12% (Faraone, Sergeant, Gillberg, & Biederman, 2003). ADHD is a biological condition resulting from a dysregulation of fronto-subcortical-cerebellar circuits (Biederman & Faraone, 2005). The symptoms of ADHD are inattention, increased hyperactivity and/or impulsivity that begin in childhood and cause a significant impairment in social and school functioning (American Psychiatric Association, 2000). Heritability of ADHD is high, with estimates exceeding 0.70 (Faraone et al., 2005; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010) although environmental influences, and gene-environment interactions, are likely to be involved in its development (Plomin, Owen, & McGuffin, 1994). Epidemiologic studies have found ADHD to be associated with maternal age, maternal smoking, alcohol and illicit substances use and stress during pregnancy, low birth weight and prematurity, organic pollutants (i.e., pesticides, polychlorinated biphenyls), lead, nutritional deficiencies, family adversity, and severe early deprivation (Biederman & Faraone,

2005; Sciberras, Mulraney, Silva, & Coghill, 2017; Thapar, Cooper, Eyre, & Langley, 2013).

1.3 Third section: Socio-environmental exposures

The brain receives multiple exposures throughout the lifespan, both positive and negative (**Figure 3**).

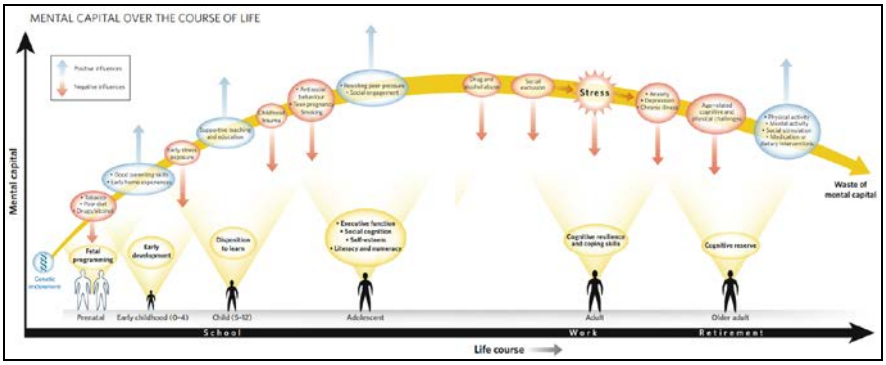

Figure 3. Mental capital over the course of life. Reproduced from Beddington et al., 2008.

These environmental influences include social and physical factors, such as parental education, parental mental health, socio-economic status or toxic agents (e.g., air pollution, alcohol or smoking) (Grandjean & Landrigan, 2014; Han et al., 2015; Tong, Baghurst, Vimpani, & McMichael, 2007).

A large number of chemicals (over 1000) are known to be neurotoxic (e.g., pesticides, organic solvents, metals and inorganic compounds) although there is an absence of chemical testing and regulations to protect society (Grandjean & Landrigan, 2006; Grandjean & Landrigan, 2014) (**Figure 4**).

Similar to genetic risk factors, the effects on causal pathways of any one environmental risk factor are small. In most cases, no single factor is necessary or sufficient to cause ND, however cumulative vulnerability and combined effects (genetic endorsement and exposure to numerous environmental risk factors) may exceed a certain threshold and cause impairment or disease (Faraone et al., 2015).

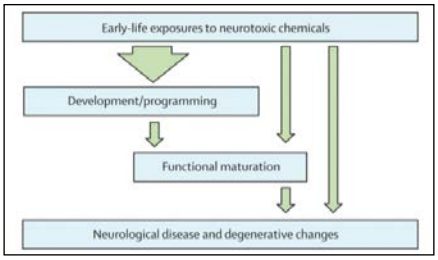

Figure 4. Effect of neurotoxicants during early brain development. Exposures in early life to neurotoxic chemicals can cause a wide range of adverse effects on brain development and maturation that can manifest as functional impairments or disease at any point in the human lifespan, from early infancy to very old age. Reproduced from Philippe Grandjean & Landrigan, 2014.

a) Traffic-related air pollution

Motor vehicles are a significant source of urban air pollution. Furthermore, the rapid growth of the world's motor-vehicle fleet due to population growth and economic improvement, the expansion of metropolitan areas, and the increasing dependence on motor vehicles has resulted in an increase in the fraction of the population living and working in close proximity to busy highways and roads ("Traffic-Related Air Pollution," 2010). From motor vehicles, there are combustion emissions (carbon dioxide (CO_2), carbon monoxide (CO), hydrocarbons (HC), nitrogen oxides (NO_x), particulate matter (PM) and mobile-source air toxics (MSATs) such as benzene, or lead) and noncombustion emissions (resuspended PM from road dust, tire wear, and break wear). Although noncombustion emissions are not regulated in the way exhaust emissions are, they both contribute to human health effects ("Traffic-Related Air Pollution," 2010). The cardiovascular and respiratory effects of air pollution have been well documented (Kaiser, 2005; Mills et al., 2009; Peters et al., 2004; Riedl, 2008). Furthermore, there is evidence of the hazards of air pollution on the CNS (Block et al., 2012) through neuroinflammation and oxidative stress (Calderón-Garcidueñas, Leray, Heydarpour, Torres-Jardón, & Reis, 2016).

Regarding traffic-related air pollution and ND, there are previous studies pointing to an association with ADHD (Fuertes et al., 2016; Perera et al., 2014), although other studies could not find any association (Abid, Roy, Herbstman, & Ettinger, 2014; Forns et al., 2016; Gong et al., 2014). For dyslexia, there is only one study, as far as we know, and no association was found (Fuertes et al., 2016).

2. RATIONALE

Substantial heritability has been reported for ND, with estimates of around 0.7. Nonetheless, the influence of environmental exposures (including physical and psychosocial factors) cannot be dismissed. Furthermore, gestation and childhood are vulnerable periods for CNS adverse effects.

There are previous reviews evaluating epidemiological evidence of the brain effects of air pollution. However, it is important to complement and update them with a synthesis of the relevant latest research results.

Prospective longitudinal studies are essential in characterizing cognitive trajectories, yet few of them have been reported on the development of attention processes in children. The study of cognitive trajectories in epidemiology can be used to identify children who deviate from normality and the identification of possible environmental or social risk factors.

Air pollution is a suspected neurodevelopmental toxicant. However, the evidence of its contribution in the development of ND such as dyslexia and ADHD is insufficient, either due to a limited number of studies or lack of consistency between studies.

Finally, there is a considerable amount of literature exploring the role of socio-environmental exposures in the development of ADHD. However, what we propose is a novel modelling framework that addresses the issue of model selection when numerous potentially correlated factors affect the outcome.

3. OBJECTIVES

The general objective of this thesis was to study the influence of socio-environmental exposures on neurodevelopmental disorders.

The specific aims were to:

1. Update previous reviews on the detrimental effects of air pollution on neuropsychological development in children.

2. Study the trajectories of attention in a large population-based cohort of primary school children.

3. Examine the acute effects of traffic-related air pollution on attention among primary school children.

4. Assess any association between pre- and postnatal exposure to traffic-related air pollution and warning signs of dyslexia in primary school children.

5. Assess independent associations between a large number of socio-environmental exposures and ADHD symptoms in preschool-aged children using a novel statistical method to deal with correlated data.

4. METHODS

4.1 Participants

a) BREATHE

The BREATHE (BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn) project, aims to assess the association between air pollution in schools and the cognition and behavior of children (Sunyer et al., 2015). This project was conducted from January 2012 to March 2013 in 36 schools in Barcelona and 3 in Sant Cugat del Vallès, a smaller city near Barcelona (Catalonia, Spain) (n=2,897). All the families of students from these 39 schools in 2nd, 3rd, and 4th primary grades were invited to participate via mail and/or project presentations in the schools. Children were evaluated in the schools every 3 months approximately over four repeated sessions using computerized neuropsychological tests.

b) INMA

The INMA (INfancia y Medio Ambiente–Environment and Childhood) project is a population-based birth cohort in Spain that aims to study the role of environmental pollutants in air, water and diet during pregnancy and early childhood in relation to child growth and development (Guxens et al., 2012). In this thesis, four regions were included: Asturias (n=365), Gipuzkoa (n=296), Sabadell (n=445), and Valencia (n=410). Pregnant women eligible for inclusion (i.e., at least 16 years of age, no communication difficulties, singleton pregnancy, no assisted fertility programme, intention to give birth at the reference hospital) were recruited at their first prenatal visit (10-13 weeks of gestation) in the main public hospital or health centre of each study area. Recruitment was done between November 2003 and February 2008.

4.2 Neuropsychological instruments

a) Attention Network Task (ANT)

The tool used to assess the attention domain was the original computerized child ANT (Rueda et al., 2004). In this version, a row of five yellow fish appearing either above or below a fixation point is presented. Children are invited to "feed" the central fish as quickly as possible by pressing either the right or the left arrow key depending on the direction in which the target fish is pointing while ignoring the flanker fish, which point in either the same (congruent) or opposite (incongruent) direction than the middle fish. The target is preceded by visual signals that inform either about the upcoming of the target only (alerting cue) or about the upcoming of the target as well as its location (orienting cue). Each correct answer is followed by a simple animation sequence (the target fish blowing bubbles) and a recorded sound ("woo hoo!"). Incorrect responses are followed by a single tone and no animation of the fish. A session of the ANT consisted of 16 practice trials and four experimental blocks of 32 trials in each (128 trials in total). Each trial represented one of 8 conditions in equal proportions: two Flanker Congruency (congruent and incongruent) × four Cue Type (no cue, central cue, double cue and spatial cue).

b) N-Back task

In the n-Back task the subjects are required to monitor a series of stimuli presented in the centre of the laptop's screen and they have to respond whenever a given stimulus is the same as the one presented n trials previously (1-, 2-, and 3-back) (Nelson et al., 2000). These different conditions are known as loads and in the highest cognitive load (i.e., 3-back) the demands on working memory are stronger. Stimuli are presented in a fixed central location on a white background for a 1500-ms duration with a 1000-ms interstimulus interval. All participants are required to press a specific keyboard button when the target appear in the screen. Participants complete three levels (1-, 2-, and 3-back) for each stimulus. In the 1-back level, the target is any stimulus that match the stimulus immediately preceding it. In the 2-back level, the target is any stimulus that match the one presented two trials previously.

In the 3-back level, the target is any stimulus identical to the presented three trials previously. Each level consists of 25 trials. The first three trials of each level are never targets, and 33% of stimuli of the following trials are targets. After each level, a short break (5–20 s) is provided to allow participants some rest. Upon completion of each target, children hear a motivational recorded sample ("woo hoo!") and a smiling face appears at the top left of the screen.

c) ADHD-DSM-IV

The ADHD Criteria of *Diagnostic and Statistical Manual of Mental Disorders, fourth Edition* (ADHD-DSM-IV) (American Psychiatric Association, 2000) form list consists of 18 symptoms categorized in two separate symptom groups (inattention and hyperactivity/impulsivity) with nine symptoms each. Teachers rated each ADHD symptom on a 4-point scale of frequency from never or rarely (0) to very often (3) for each child, and subscales can therefore be scored from 0 to 27 and the global score can range from 0 to 54. Higher scores indicate higher symptomatology. We also used a categorical variable of ADHD clinical criteria with four categories, according to the presence of 6 or more symptoms of each subtype: no ADHD; ADHD-inattentive; ADHD-hyperactive/impulsive; and ADHD-combined. For that, we recoded options 0 and 1 as 0 (symptom absent), and options 2 and 3 as 1 (symptom present) (Gomez, 2007).

d) PRODISCAT

Warning signs of dyslexia in primary school age were reported by the classroom teachers through a 3-4 items questionnaire. The items were taken from the PRODISCAT: *Protocol de detecció i actuació en la dislèxia. Àmbit educatiu* (Protocol for detection and management of dyslexia. Educational scope), the current official Catalonian protocol (Col·legi de Logopedes de Catalunya, 2011). PRODISCAT has six versions that correspond to the different educational levels. We used version 2 and 3 (1st - 2nd grades, and 3rd - 4th grades, respectively).

The three Yes/No survey questions for 2nd grade students were (1) "Has difficulties in the grapheme-phoneme correspondence", (2)

"Has difficulties in sounding out words", (3) "Commits many spelling errors compared to the group". The four survey questions for 3rd and 4th grade students, were (1) "Commits many reading errors including: omissions, substitutions, additions and transpositions", (2) "Has difficulties in reading comprehension (due to poor accuracy)", (3) "Commits many spelling mistakes compared to the group", (4) "Has difficulties in sequencing (months, seasons, alphabet…)".

4.3 Exposure assessment

a) Traffic-related air pollution in schools

We measured short-term exposures based on the daily ambient levels of nitrogen dioxide (NO_2) and elemental carbon (EC) in particulate matter <2.5 μm ($PM_{2.5}$) filters; measures were taken at a fixed air quality background monitoring station in Barcelona operating continuously throughout the year. In addition, we measured long-term school exposure based on average indoor pollution levels in classrooms, using direct measurements conducted in schools in days not coinciding with the outcome assessment that cover the entire year 2012. We measured each pair of schools simultaneously during four complete days (from Monday to Friday) on two occasions 6 months apart, during the warm and cold periods of the year 2012. We measured indoor air in a single classroom and outdoor air in the courtyard simultaneously.

b) Traffic-related air pollution at the home residence

We selected NO_2 as a surrogate for road traffic emissions given its relation to vehicle exhaust emissions in Barcelona, a diesel-dominated vehicle fleet (with very high NO_2 emissions) (Henschel et al., 2015; Rivas et al., 2014). We used Land Use Regression models (LUR) for Barcelona developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) (Beelen et al., 2013; Eeftens et al., 2012). LUR models are based on temporally-adjusted spatial estimates of air pollutant levels. Several potential predictors (e.g., population density, land use and traffic-related variables) derived from geographic information systems (GIS) are

used to explain within-city spatial variability in traffic-related air pollutants concentrations (Eeftens et al., 2012). We estimated the average concentration of NO_2 at the residence addresses of the prenatal period and the postnatal period (the first 6 years of life) using LUR for each participant. To temporally adjust them to each specific period we used the daily standardized measurements from the air quality national network (XVPCA, http://dtes.gencat.cat/icqa).

c) Exposures in INMA cohorts

The information was collected from a variety of sources: ad hoc interviewer-administered questionnaires by trained INMA personnel, clinical data, physical examinations, biological samples (urine, blood), diet determinants and environmental measurements. The following factors obtained from first trimester questionnaire were explored in the study analysis: parental age, parental education, parental social class, parental country of birth, maternal pre-pregnancy body mass index, parity, and marital status. The following factors were included from the third trimester questionnaire: folic acid supplementation, seafood consumption, weight gain during pregnancy, maternal smoking, alcohol and paracetamol use, maternal environmental tobacco smoke exposure, and maternal mental health. Breastfeeding was assessed when children were six and 14 months old. The following covariates were included from the follow-up at age 4-5 years: a proxy of maternal verbal IQ, parental mental health, use of gas appliances at home, and child environmental tobacco smoke exposure. Several biomarkers were measured including maternal vitamin D concentrations, maternal and child cotinine levels, and mercury concentrations from cord blood. Information related to the child's birth, including date, gestational age, gender, and birth anthropometry were available from clinical records. Finally, data on outdoor air pollution (NO_2, benzene) throughout the pregnancy was obtained using LUR models.

5. RESULTS

Paper I. Air Pollution and Neuropsychological Development: A Review of the Latest Evidence

Paper II. A Longitudinal Study on Attention Development in Primary School Children with and without Teacher-Reported Symptoms of ADHD

Paper III. Traffic-related Air Pollution and Attention in Primary School Children. Short-term Association

Paper IV. Traffic-related air pollution and signs of dyslexia in primary schoolchildren

Paper V. Socio-environmental exposures associated with teacher-reported symptoms of ADHD in preschoolers

5.1 Paper I

Suades-González E, Gascon M, Guxens M, Sunyer J. Air pollution and neuropsychological development: A review of the latest evidence. Endocrinology. 2015 Oct;156(10):3473-82. DOI: 10.1210/en.2015-1403.

5.2 Paper II

Suades-González E, Forns J, García-Esteban R, López-Vicente M, Esnaola M, Álvarez-Pedrerol M, et al. A longitudinal study on attention development in primary school children with and without teacher-reported symptoms of ADHD. Front Psychol. 2017 May 16;8:655. DOI: 10.3389/fpsyg.2017.00655.

5.3 PAPER III

Sunyer J, Suades-González E, García-Esteban R, Rivas I, Pujol J, Alvarez-Pedrerol M, et al. Traffic-related Air Pollution and Attention in Primary School Children. Epidemiology. 2017 Mar 1;28(2):181–9. DOI: 10.1097/EDE.0000000000000603

5.4 Paper IV

Suades-González E, García-Esteban R, Forns J, Rivas I, Cirach M, López-Sala A, Sunyer J. Traffic-related air pollution and signs of dyslexia in primary schoolchildren. *Submitted to Environmental Research.*

Traffic-related air pollution and signs of dyslexia in primary schoolchildren

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ABSTRACT

Introduction: Few studies have examined the role of the environmental influences in the development of dyslexia. We aim to explore whether pre- and postnatal exposure to traffic-related air pollution is associated with warning signs of dyslexia in primary school children.

Methods: The population sample included 2,226 children (49% girls) aged 7-11 years from 39 schools in Barcelona (Spain). Warning signs of dyslexia were assessed using a questionnaire for teachers. Average concentration of NO_2 at the residence address of the pre- and the postnatal periods (first 6 years of life) were estimated for each participant. Air pollution concentration was categorized into quartiles. Models were adjusted by a number of covariates, including ADHD symptoms.

Results: For prenatal exposure, in 3rd and 4th grades students, we observed an increase in signs of dyslexia in children in the 2nd exposure quartile (RR = 1.52, 95% CI 1.07–2.15) and 3rd quartile (RR = 1.39, 95% CI 1.02–1.89) compared to those in the lowest quartile, while the association became more flat in children in the 4th quartile compared to those in the lowest. This association was not observed in children in the 2nd grade. For postnatal exposure, similar results were observed although the point estimates were lower, the confidence intervals were wider and included the null value.

Conclusions: Prenatal, and at some extent postnatal, exposure to NO_2 at the residence address increased the risk of presenting signs of dyslexia in primary school children.

Keywords: Dyslexia, air pollution, NO_2, children.

INTRODUCTION

Dyslexia refers to a specific reading disability despite educational opportunity and adequate intelligence. It is one of the most common neurodevelopmental disorders with a prevalence rate of around 7% (Peterson & Pennington, 2012). The neural correlates of dyslexia across languages (white matter changes in posterior and anterior left hemisphere language networks) are well documented (Peterson & Pennington, 2012). There is evidence for its high heritability rates (approximately 75%) although the influence of environmental factors, and their interplay with genetics, cannot be dismissed (Olson, 2006; Peterson & Pennington, 2012). Previous literature pointed out that family socioeconomic status (e.g., parents' education level, language and literacy environment) (Olson, 2006; Peterson & Pennington, 2012), and child health risks (e.g., gestational age) (Kovachy, Adams, Tamaresis, & Feldman, 2015) are the main environmental determinants of dyslexia. However, much remains to be learned about the role of the environment in its development. It is in this context that we raise the issue of whether environmental toxins could be involved in the development of dyslexia. Among the existing environmental toxins, air pollution is known to affect the central nervous system through neuroinflammation and oxidative stress (Calderón-Garcidueñas, Leray, Heydarpour, Torres-Jardón, & Reis, 2016), and structural brain alterations include white matter injury (Babadjouni et al., 2017). Fetal life and early childhood are vulnerable periods of brain development (Rice & Barone, 2000). Dyslexia is a developmental learning disorder and probably as a result of prenatal, and to a lesser extent early postnatal, abnormal brain maturation due to genetic and intrauterine environmental influences (Gilger & Kaplan, 2001). Furthermore, preschool years are crucial for development of the core *cognitive* process underlying reading acquisition, the phonological processing (i.e., phoneme awareness) (Carroll, Snowling, Hulme, & Stevenson, 2003; Melby-Lervåg, Lyster, & Hulme, 2012). For the last decade, there has been mounting evidence of the detrimental impact of air pollution on cognitive functioning (Clifford, Lang, Chen, Anstey, & Seaton, 2016; Suades-González, Gascon, Guxens, & Sunyer, 2015) although studies on air pollutants exposure and learning disabilities is still scarce. Clark et al. (2012) did not find any association between postnatal exposure to NO_2 at school and reading comprehension, or other cognitive

outcomes, in UK children aged 9–10. In a large birth cohort study conducted in Germany, the authors reported no association between postnatal traffic-related air pollution exposure and dyslexia or dyscalculia but only for hyperactivity/inattention scores (Fuertes et al., 2016).

The aim of this study was to assess any association between the exposure during pre- and postnatal periods to traffic-related air pollution estimated at the residence address and teacher-reported warning signs of dyslexia in primary school children. This would be the first time to account for in utero exposure, in addition to the postnatal period.

METHODS

Study population: This study is part of the BREATHE (BRain dEvelopment and Air polluTion ultrafine particles in scHool childrEn) project, a longitudinal study conducted in 39 schools in Barcelona (Spain) from January 2012 to March 2013. All students without special needs were recruited and a total of 2,897 subjects in 2nd, 3rd, and 4th primary grades participated in the study (59% response rate). Further details on study settings and school selection process can be found elsewhere (Sunyer et al., 2015).

From the initial sample, 172 children (5.9%) were excluded due to missing data on the outcome and 499 (17.2%) due to exposure missing data (i.e., adopted, foreign-born, or with missing data on more than 25% of one year due to difficulties in geocoding addresses reported by the parents).

The final sample size included in the study was of 2,226 children (48.8% girls) aged from 7 to 11 years (Mean = 8.5; SD = 0.87). **Table 1** shows the characteristics of the included (n = 2,226) and excluded (n = 671) population, and the exposure to NO_2 during pregnancy and the first 6 years of life of the included participants.

The included participants did not differ with the excluded by grade, gender, date of birth, birth weight, gestational age, smoking during pregnancy or socioeconomic vulnerability. However, included children were slightly younger than the excluded (8.45 vs. 8.55 respectively, $p = 0.013$), a lower proportion had a foreign origin (6.18%, $p < 0.001$), were breastfed (16.29%, $p < 0.001$) and presented teacher-rated ADHD symptoms (9.71%, $p = 0.032$). On the contrary, a higher proportion of the included had older siblings (46.14%, p = 0.006), parents with a university degree ($p < 0.005$)

and Catalan or bilingual context at home (51.46% and 13.79% respectively, $p < 0.001$).
All parents or guardians signed the informed consent form approved by the Clinical Research Ethical Committee (No. 2010/41221/I) of the Institut Hospital del Mar d'Investigacions Mèdiques.

Exposure: We estimated exposure to traffic-related air pollution during pre- and postnatal (up to 6 years of age) periods at the residence address. Among the different air pollutants, we selected nitrogen dioxide (NO_2) as a surrogate for road traffic emissions given its relation to vehicle exhaust emissions in Barcelona, a diesel-dominated vehicle fleet (with very high NO_2 emissions) (Henschel et al., 2015; Rivas et al., 2014). We used Land Use Regression models (LUR) for Barcelona developed as part of the European Study of Cohorts for Air Pollution Effects (ESCAPE) project (Beelen et al., 2013; Eeftens et al., 2012). LUR models are based on temporally-adjusted spatial estimates of air pollutant levels. Several potential predictors (e.g., population density, land use and traffic-related variables) derived from geographic information systems (GIS) are used to explain within-city spatial variability in traffic-related air pollutants concentrations (Eeftens et al., 2012). We estimated the average concentration of NO_2 at the residence addresses of the prenatal period and the postnatal period (the first 6 years of life) using LUR for each participant. To temporally adjust them to each specific period we used the daily standardized measurements from the air quality national network (XVPCA, http://dtes.gencat.cat/icqa). History of residence was reported by the parents through a questionnaire indicating dates and address. In case of changes in residence (33.5% of families), we calculated the time-weighted average of the air pollutants estimated for each address. For divorced or separated parents with shared custody (15.2% of families), time averaged exposure was calculated according to time spent in each residence.

Outcome: Warning signs of dyslexia in primary school age were reported by the classroom teachers through a 3-4 items questionnaire. The items were taken from the PRODISCAT: *Protocol de detecció i actuació en la dislèxia. Àmbit educatiu* (Protocol for detection and management of dyslexia. Educational scope), the current official Catalonian protocol (Col·legi de Logopedes de Catalunya, 2011). PRODISCAT has six versions that

correspond to the different educational levels. We used version 2 and 3 (1st - 2nd grades, and 3rd - 4th grades, respectively).
The three Yes/No survey questions for 2nd grade students were (1) "Has difficulties in the grapheme-phoneme correspondence", (2) "Has difficulties in sounding out words", (3) "Commits many spelling errors compared to the group". The four survey questions for 3rd and 4th grade students, were (1) "Commits many reading errors including: omissions, substitutions, additions and transpositions", (2) "Has difficulties in reading comprehension (due to poor accuracy)", (3) "Commits many spelling mistakes compared to the group", (4) "Has difficulties in sequencing (months, seasons, alphabet…)".
Signs of dyslexia were analyzed as a general score by grade (0-3 for 2nd grade, 0-4 for 3rd and 4th grade).

Covariates: Contextual and individual socio-demographic characteristics were obtained using a neighbourhood socioeconomic status vulnerability index (Ministry of Public Works (Spain), 2001) (based on level of education, unemployment, and occupation in the census tract at the residence address) (Sunyer et al., 2015) as well as through a questionnaire completed by parents (e.g., date of birth, gender, birth weight, gestational age, family origin, parental education, linguistic context at home). Attention deficit hyperactivity disorder (ADHD) symptoms were reported by teachers filling out the ADHD Criteria of *Diagnostic and Statistical Manual of Mental Disorders, fourth Edition* (ADHD-DSM-IV) list (American Psychiatric Association, 2000). NO_2 concentrations measured in the school yard at the time of the exam were described elsewhere (Sunyer et al., 2015).

Statistical analysis: Poisson mixed effects models including school as random effect were used to address the multilevel nature of the data. The results are presented as rate ratios (RR) with corresponding 95% confidence intervals (95% CI). Generalized additive mixed models (GAMM) were used to evaluate the linearity of the relationship between air pollution exposure and dyslexia score (**Figure S1**). In subsequent models, air pollution concentration was categorized into quartiles.
Models were adjusted for variables selected a-priori as described elsewhere (Sunyer et al., 2015). The selected variables were: age, gender, maternal education (primary or less/secondary/university),

residential neighborhood socioeconomic status and teacher-rated ADHD symptoms. Stratified analyses were performed by grade.

We conducted sensitivity analysis to explore the potential for residual confounding by variables a-priori related to dyslexia: birth weight, gestational age (preterm), and linguistic context at home (Kovachy et al., 2015; Lundberg, 2002). Moreover, to control for other exposures we also adjusted for smoking during pregnancy and outdoor school air pollution exposure. Furthermore, analyses were performed excluding children with teacher-rated ADHD symptoms due to the high comorbidity between dyslexia and ADHD (Willcutt & Pennington, 2000). Finally, given preliminary evidence that males might be more susceptible than females to air pollution neurotoxicity (Clifford et al., 2016; Suades-González et al., 2015) we stratified analysis by gender.

We used inverse probability weighting (IPW) to account for potential selection bias that arises when only children with available exposure and outcome data are included as compared to the initial children recruited (**Table 1**). Briefly, we used information for all children at recruitment to predict the probability of participation in the study, and used the inverse of those probabilities as weights in the analysis so that results would be representative for the initial population. The variables used to calculate weights are described in **Table S1** in the Supplemental Material. We obtained a good fit of the participation model based on the area under the Receiver Operating Characteristic curve (AUROC = 0.8412).

Statistical analyses were done using Stata 14.2 (Stata Corporation, College Station, Texas) and R (3.2.5; R Foundation for Statistical Computing).

RESULTS

Children were on average 8.5 years old and 48.8% were girls. Maternal education level was high (60% of mothers had a university degree). According to the questionnaires rated by the teachers 9.7% of children presented ADHD symptoms. Average NO_2 concentrations during the pregnancy period compared to the postnatal period (6 first years) were similar (52.52 μg m^3 and 51.09 μg m^3, respectively) and highly correlated (Spearman's rho = 0.880, $p < 0.001$) (**Table 1**).

Table 2 shows the percentages of participants with signs of dyslexia by grade, gender and teacher-rated ADHD symptoms. No signs of

dyslexia were found in 78.6% of 2nd grade students, 72.5% in 3rd grade and 68.1% in 4th grade. The item related to spelling mistakes was the highest rated across grades in students with signs of dyslexia (**Table S2**). Signs of dyslexia were more present in males and children with symptoms of ADHD.

Main analysis

Changes in signs of dyslexia scores associated with traffic-related air pollutants by grade are presented in **Table 3**. No associations were encountered in 2nd grade students (**Table 3**). Prenatal exposure to NO_2 increased the risk of presenting warning signs of dyslexia in 3rd and 4th grade students. Specifically, those in the 2nd exposure quartile increased about 50% the number of signs of dyslexia compared to those in the lowest quartile (RR = 1.52, 95% CI 1.07–2.15) and students in the 3rd quartile increased about 40% the number of signs of dyslexia compared to those in the lowest quartile (RR = 1.39, 95% CI 1.02–1.89). We found a marginal association for postnatal exposure in the main model.

Sensitivity analysis

Further adjustment for other covariates (i.e., birth weight, gestational age, linguistic context at home, smoking during pregnancy, and outdoor school NO_2 concentrations) did not change the associations observed in our main analysis. After excluding children with teacher-rated ADHD symptoms (n=227 (10.2%)) we found a stronger association for the prenatal period (Q_2 vs Q1: RR = 1.65, 95% CI 1.10–2.48; Q_3 vs Q_1: RR = 1.53, 95% CI 1.07–2.19) and an association for the postnatal period (RR = 1.73, 95% CI 1.15–2.62 for 2nd vs 1st quartile; RR = 1.42, 95% CI 1.01–2.00 for 4th vs 1st quartile) (**Figure 1**). The population sample after excluding children with ADHD symptoms did not differ from the initial sample except for percentage of girls (51.5% vs 48.8%, respectively).

In stratified analysis by gender, the association was stronger in girls though it was not statistically significantly different from the association in boys (*p* for interaction > 0.1) (**Figure 1**).

DISCUSSION

Prenatal exposure to traffic-related air pollution (NO_2) estimated at the residence address was associated with warning signs of dyslexia in primary-school children. The magnitude of this association was weaker for postnatal exposure.

Dyslexia tends to run in families although the role of the environmental influences needs also to be considered (Fisher & DeFries, 2002). There is previous literature investigating the contribution of socioeconomic status (e.g., parental education, home literacy environment) or child health (e.g., prematurity) risk factors to reading difficulties (Dilnot, Hamilton, Maughan, & Snowling, 2017; Kovachy et al., 2015). However, the role of developmental neurotoxicants such as air pollution (Grandjean & Landrigan, 2014) has barely been considered as predisposing risk factors for dyslexia, a disorder that is neurobiological in origin. In the present study, we indeed found a higher risk for signs of dyslexia associated to traffic-related air pollution in the elder groups. No association was observed in the youngest children which could be related to a lower sample size as well as a lower percentage of participants with signs of dyslexia compared to 3rd and 4th grades. Indicators of dyslexia can be detected from preschool to adulthood although they differ across the age groups; this was indeed taken into account in the protocol used (PRODISCAT). However, reading difficulties in the first years of primary school can be often justified by the teachers as a developmental lag instead of the presence of warning signs of dyslexia.

To our knowledge, there are no previous epidemiological studies analyzing the association between exposure to traffic pollution during the pregnancy period and signs of dyslexia. Nonetheless, a study conducted in Germany did investigate the associations with dyslexia and postnatal exposure (until age 15 years) to traffic-related air pollution and obtaining no associations (Fuertes et al., 2016). In our study, due to high collinearity between pre- and postnatal exposure at the residence address, it was not possible to distinguish a critical period of exposure, although we found stronger associations at the prenatal compared to the postnatal period, and adjustment by current exposure at school did not change the associations. Furthermore, dyslexia is a developmental learning disorder and probably as a result of prenatal, and to a lesser extent early postnatal, abnormal brain maturation due to genetic and

intrauterine environmental influences (Gilger & Kaplan, 2001). In fact, Galaburda et al. (1985) referred to the neuroanatomical findings seen in dyslexia (i.e., neuronal ectopias and architectonic dysplasias) as acquired during the middle of gestation, a period with peak rates of neuronal migration from the germinal zones to the cerebral cortex. These findings have been subsequently supported by animal models (Platt et al., 2013).

Regarding potential gender differences, the difference between boys and girls was not statistically significant, although the magnitude of the effect was more pronounced in females. In relation to this, Harlaar, et al. (2005) found genetic influences to be more important as a cause of reading difficulties, and therefore a lower role for environmental factors, in boys compared to girls. However, subsequent studies could not replicate these findings (Wadsworth & DeFries, 2005) and further research is warranted.

This study has some strengths and limitations that need to be considered. We assessed exposure at the residence overlooking at the contribution of other sources (e.g., outdoor exposure at work (during pregnancy), indoor air pollution levels at home/work/school, or exposure during commuting). Nevertheless the exposure at home contributes by around 50-60% of the total air pollution exposure (Rivas et al., 2016). Furthermore, the association remained after additional adjustment for school exposure and smoking during pregnancy, minimizing a potential residual confounding explanation. Moreover, we cannot rule out the likelihood of residual confounding by socio-demographic confounding; however, we adjusted our analyses for indicators of individual (i.e., maternal education) and residential neighborhood socioeconomic status which should have minimized such likelihood. Regarding the outcome, the teacher-rated signs of dyslexia used in this study are not to be confounded with a clinical diagnosis of dyslexia by a qualified professional. In addition, the validity of the outcome seemed poorer at the youngest age group. Another limitation is the potential residual confounding by ADHD, a disorder with high rates of comorbidity with dyslexia (Willcutt & Pennington, 2000). Furthermore, there are previous studies pointing to an association with traffic-related air pollution exposure and ADHD or ADHD symptomatology (Fuertes et al., 2016; Perera et al., 2014), although other studies could not find any association (Abid, Roy, Herbstman, & Ettinger, 2014; Forns et al., 2016; Gong et al., 2014). However, after repeating the analysis excluding

children with teacher-rated ADHD symptoms, the results did not change remarkably but confirmed the associations found in the main model. The higher rate of girls in the sample after excluding children with ADHD symptoms could explain the stronger associations found in the pre- and postnatal exposure. Strengths of this population-based study include the large sample size, the use of a standardized air pollution assessment, and the adjustment of models for many variables that are known to be associated with dyslexia.

Conclusions

We observed an association between prenatal, and at some extent postnatal, exposure to traffic-related air pollution and warning signs of dyslexia. Learning disabilities may affect children's academic achievement and potentially diminish the mental capital on societies. The public health impact cannot be ignored and policies on air quality are urgently required to protect children.

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Table 1. Description of the included and the excluded population from BREATHE participants.

Characteristic	included	excluded	*p*-value[a]
Number of children (n)	2226	671	
Age at session 1 (mean, SD)	8.45 (0.87)	8.55 (0.88)	0.013
Grade (%)			0.262
2nd	38.19	34.72	
3rd	35.22	37.56	
4th	26.59	27.72	
Gender (% girls)	48.79	52.91	0.061
Older siblings (%)	46.14	39.72	0.006
Adopted (%)	0	18.20	<0.001
Foreign origin (%)	6.18	46.41	<0.001
Date of birth (% Q4)	25.61	24.49	0.687
Birth weight, < 2.5 kg (%)	9.35	10.76	0.337
Gestational age, < 37 weeks (%)	7.40	8.45	0.428
Smoking during pregnancy (%)	9.95	9.27	0.647
Maternal education (%)			0.002
Primary or less	11.69	16.70	
Secondary	28.35	29.88	
University	59.95	53.43	
Paternal education (%)			0.012
Primary or less	14.56	19.67	
Secondary	31.79	30.78	
University	53.65	49.54	
Linguistic context at home (%)			<0.001
Catalan	51.46	35.43	
Spanish	28.47	40.49	
Bilingual	13.79	10.82	
Other languages	6.27	13.26	
ADHD symptoms -DSM-IV, teachers form[b] (%)	9.71	12.66	0.032
Home socioeconomic vulnerability index (mean, SD)	0.45 (0.21)	0.46 (0.21)	0.184
Residential NO_2 concentrations ($\mu g/m^3$)			
During pregnancy	52.52		
Mean (SD)	(16.84)	-	-
25th percentile	43.72		
Median	52.06		
75th percentile	59.30		

Postnatal exposure (6 first years)			
Mean (SD)	51.09 (15.77)	-	-
25th percentile	42.95		
Median	51.12		
75th percentile	57.01		

[a] Mann-Whitney test for continuous variables and Chi-square tests for categorical variables.

[b] *ADHD Criteria of Diagnostic and Statistical Manual of Mental Disorders, fourth edition. Teachers form.*

Q4, Quartile 4: Children born from October 1 to December 31.

Table 2. Number of participants (N, %) with signs of dyslexia by grade, gender and teacher-rated ADHD symptoms.

	Number of signs of dyslexia				
	0	1	2	3	4
Grade					
2nd	659 (78.55)	83 (9.89)	41 (4.89)	56 (6.67)	_
3rd	558 (72.47)	77 (10.00)	47 (6.10)	50 (6.49)	38 (4.94)
4th	399 (68.09)	78 (13.31)	49 (8.36)	36 (6.14)	24 (4.10)
Gender					
Male	793 (70.74)	142 (12.67)	71 (6.33)	75 (6.69)	40 (3.57)
Female	823 (76.63)	96 (8.94)	66 (6.15)	67 (6.24)	22 (2.05)
Teacher-rated ADHD symptoms					
No	1531 (77.64)	195 (9.89)	113 (5.73)	95 (4.82)	38 (1.93)
Yes	75 (35.55)	42 (19.91)	24 (11.37)	47 (22.27)	23 (10.90)

2nd grade, 7-8 years old; 3rd grade, 8-9 years old; 4th grade, 9-10 years old.

_ Not applicable.

Table 3. Changes (Rate Ratio, 95% CI)† in signs of dyslexia scores associated with traffic-related air pollution by grade.

	NO_2					
	Prenatal			Postnatal		
	N	RR (95% CI)	*p*	N	RR (95% CI)	*p*
2nd grade						
Q_1	219	ref.		178	ref.	
Q_2	235	1.17 (0.85, 1.61)	0.324	192	1.02 (0.70, 1.48)	0.924
Q_3	196	0.96 (0.62, 1.50)	0.860	221	1.18 (0.84, 1.67)	0.338
Q_4	166	0.86 (0.51, 1.44)	0.564	223	0.98 (0.65, 1.49)	0.937
3rd-4th grade						
Q_1	305	ref.		342	ref.	
Q_2	292	1.52 (1.07, 2.15)	0.019	338	1.44 (0.96, 2.15)	0.080
Q_3	343	1.39 (1.02, 1.89)	0.034	310	1.22 (0.84, 1.79)	0.300
Q_4	356	1.27 (0.93, 1.73)	0.134	303	1.26 (0.83, 1.92)	0.269

RR, Rate Ratio; CI, Confidence Interval; Q_i, Quartiles.
† Coefficients obtained from Poisson analysis adjusted for age, gender, maternal education, residential neighborhood socioeconomic status and teacher-rated ADHD symptoms; school as random effect.

Figure 1. Sensitivity analysis and stratified analysis of the association (Rate Ratio and 95% Confidence Intervals) between traffic-related air pollution (NO_2, $\mu g/m^3$) and signs of dyslexia scores in 3rd-4th grade students.

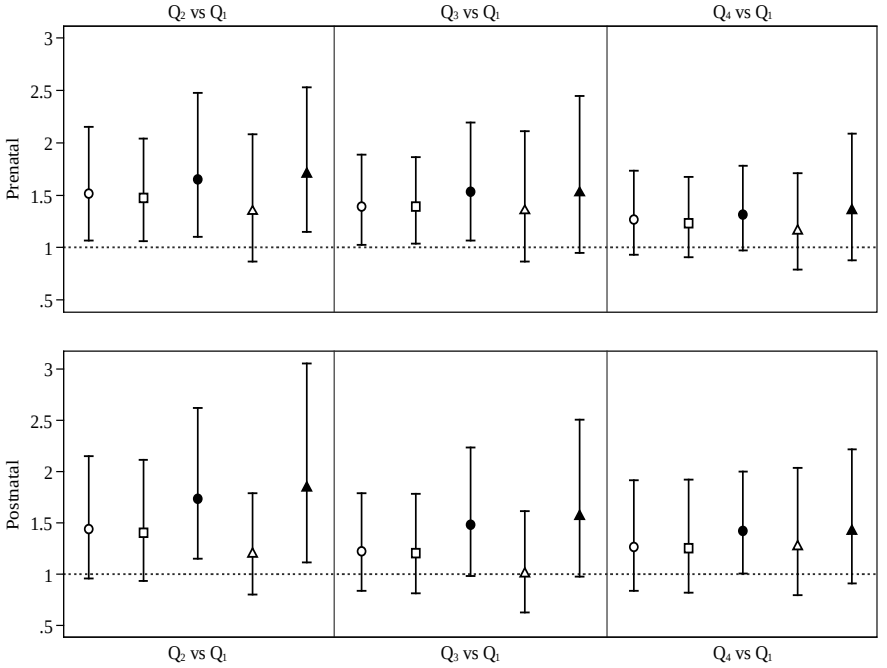


Figure legend

○: Main model. Adjusted for gender, age, maternal education, residential neighborhood socioeconomic status, and teacher-rated ADHD symptoms as fixed effects; and school as random effect.

□: Main model also adjusted for birth weight, preterm, linguistic context at home, smoking during pregnancy, and outdoor school air pollution exposure.

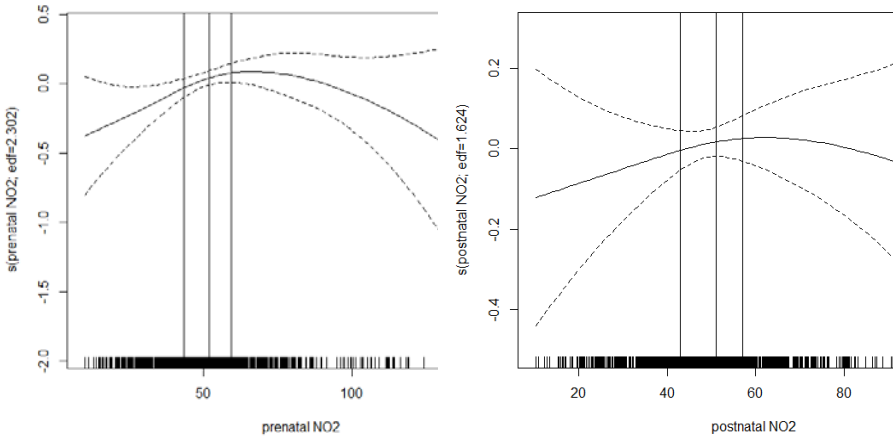
●: Main model among children without ADHD symptoms (N obs.=1173)

△: Main model among boys (N obs.=657)

▲: Main model among girls (N obs.=639)

Supplementary material

Figure S1. Relationship between pre- and postnatal exposure to traffic-related air pollution (NO_2, $\mu g/m^3$) and signs of dyslexia among children in 3rd and 4th grade. Vertical lines represent the quartile cut-offs.



GAMM models with family Poisson adjusted for age, gender, maternal education, residential neighborhood socioeconomic status as fixed effects, and school as random effect. The y-axis shows the additive effect on the log- of dyslexia symptoms when using a smooth function on NO_2; edf. refers to estimated degrees of freedom of the curve.
The relationship was estimated linear (edf=1) among children in 2nd grade.

Table S1. List of potential covariates for the participation model.

Explored variables	Included*
Age	x
Degree	x
Gender	
Older siblings	x
Ethnicity	x
Quarter of birth date	
Birth weight < 2.5 kg	
Gestational age < 37 weeks	
Breastfeeding	
Obesity	
Maternal smoking during pregnancy	
Maternal educational level	
Paternal educational level	
Linguistic context at home	
Teacher-rated ADHD symptoms	x
SDQ score	x
Residential neighborhood socioeconomic status	x
NDVI at birth address	x

* Selected by using forward selection

Table S2. Description of the survey questions in participants with signs of dyslexia by grade.

	Positive answer		Negative answer	
	N	%	N	%
2nd grade				
Item 1	92	48,42	98	51,58
Item 2	88	48,35	94	51,65
Item 3	154	88,91	34	18,09
3rd and 4th grade				
Item 1	201	48,55	213	51,45
Item 2	248	60,19	164	39,81
Item 3	335	80,92	79	19,08
Item 4	96	23,70	309	76,30

Excluded subjects due to no signs of dyslexia: 2nd grade N=659, 3rd and 4th grade N=957.
Items description by grade. 2nd grade: Item 1, "Has difficulties in the grapheme-phoneme correspondence", Item 2, "Has difficulties in sounding out words", Item 3, "Commits many spelling errors compared to the group". 3rd and 4th grade: Item 1, "Commits many reading errors including: omissions, substitutions, additions and transpositions", Item 2, "Has difficulties in reading comprehension (due to poor accuracy)", Item 3, "Commits many spelling mistakes compared to the group", Item 4, "Has difficulties in sequencing (months, seasons, alphabet…)".

5.5 PAPER V

Suades-González E, Forns J, García-Esteban R, Andiarena A, Guxens M, Ibarluzea J, Riaño Galan I, Tardón A, Vrijheid M, López-Sala A, Sunyer J. Socio-environmental exposures associated with teacher-reported symptoms of ADHD in preschoolers.

Socio-environmental exposures associated with teacher-reported symptoms of ADHD in preschoolers

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ABSTRACT

Introduction: The aim of this study was to assess the independent association between socio-environmental exposures (including psychosocial and physical factors) and ADHD symptoms in preschool-aged children using a novel statistical method to deal with correlated data.

Methods: We used data from the INMA – Infancia y Medio Ambiente (Environment and Childhood) project. We analyzed 23 socio-environmental factors related to ADHD (attention deficit hyperactivity disorder) in a birth cohort of 1,516 children. We assessed ADHD symptomatology at age 5 years using ADHD-DSM-IV form list completed by the teachers. The deletion/substitution/addition (DSA) algorithm was employed to select a model for ADHD (global), Inattention and Hyperactivity/Impulsivity symptoms scores, and by gender.

Results: We found male gender (RR = 1.67, 95% CI 1.50–1.87), younger relative age (RR = 1.58, 95% CI 1.35–1.85), low social class (RR = 1.18, 95% CI 1.05–1.33), maternal depression (RR = 1.20, 95% CI 1.00–1.45), paternal hostility (RR = 1.21, 95% CI 1.05–1.41), environmental tobacco smoke (RR = 1.04, 95% CI 1.02–1.07), and prenatal alcohol consumption (RR = 1.04, 95% CI 1.01–1.07) to be positively associated with ADHD symptoms score in a birth cohort of preschool children. ADHD inattentive symptoms score showed more associations with socio-environmental factors compared to ADHD hyperactive/impulsive symptoms score. By gender, psychosocial factors showed a stronger association with ADHD symptoms score in boys, and prenatal exposure to toxics showed a stronger association with ADHD symptoms score in girls.

Conclusion: Among a total of 23 socio-environmental factors related to ADHD, only male gender, younger relative age, psychosocial adversity, smoking and prenatal alcohol consumption found to be associated with ADHD symptoms at preschool age using a novel method for handling correlated data.

Keywords: ADHD, attention deficit hyperactivity disorder, preschool children, environmental exposures, environment.

INTRODUCTION

Attention deficit hyperactivity disorder (ADHD) is one of the most common neurodevelopmental disorders in children, with an estimated prevalence ranging from 8 to 12% (Stephen V. Faraone, Sergeant, Gillberg, & Biederman, 2003). It is a biological condition resulting from a dysregulation of fronto-subcortical-cerebellar circuits (Biederman & Faraone, 2005). The symptoms of ADHD are inattention, increased hyperactivity and/or impulsivity that begin in childhood and cause a significant impairment in social and school functioning (American Psychiatric Association, 2000). Heritability of ADHD is high, with estimates exceeding 0.70 (Stephen V. Faraone et al., 2005; Lichtenstein, Carlström, Råstam, Gillberg, & Anckarsäter, 2010). However, the genetics of ADHD are complex due to its multigenic etiology and only candidate genes (neither necessary nor sufficient) with small risk for the disorder can be identified (Gizer, Ficks, & Waldman, 2009). Furthermore, environmental influences, and gene-environment interactions, are likely to be involved in the development of complex disorders accounting for some quarter to a half of the variance (Plomin, Owen, & McGuffin, 1994). However, identifying multiple environmental factors is a challenge. With regard to ADHD, pre and perinatal risks, psychosocial adversity and environmental toxins during prenatal and/or postnatal life have been reportedly as predisposing risk factors. Specifically, epidemiologic studies have found ADHD diagnosis or ADHD symptomatology to be associated with maternal age, maternal smoking, alcohol and illicit substances use and stress during pregnancy, low birth weight and prematurity, organic pollutants (i.e., pesticides, polychlorinated biphenyls), lead, nutritional deficiencies, family adversity, and severe early deprivation (Biederman & Faraone, 2005; Sciberras, Mulraney, Silva, & Coghill, 2017; Thapar, Cooper, Eyre, & Langley, 2013). There is previous literature attempting the study of pre- and perinatal risk factors associated with ADHD from a multifactorial approach (Henriksen, Wu, Secher, Obel, & Juhl, 2015; Silva, Colvin, Hagemann, & Bower, 2014) although the inclusion of the chemical environment is lacking. In this paper, we followed a naïve approach that allowed us analyse a multiple set of risk factors using methods that handle correlated data. We propose a modelling framework that addresses the issue of model selection when numerous variables jointly affect the outcome. We aimed to assess

independent associations between a large number of socio-environmental exposures (including psychosocial and physical factors) and ADHD symptoms at 5 years of age using the INMA birth cohort. We employed this novel framework to derive models for ADHD outcomes with high number of potentially correlated risk/protective factors. Furthermore, we examined whether the associations differed between ADHD symptom domains, and by gender. Differences in heritability, and therefore in environmental influences, have been reported across ADHD subtypes (Willcutt et al., 2010), and although similar family genetic risk for ADHD for boys and girls has been previously reported (Faraone et al., 2000), further clarification about environmental factors and gender-based vulnerability is warranted (Biederman, Faraone, & Monuteaux, 2002).

MATERIALS AND METHODS

Study population: This study was based on four population-based birth cohorts established in Valencia, Sabadell, Asturias and Gipuzkoa, Spain, as part of the INMA (INfancia y Medio Ambiente –Environment and Childhood) project (Guxens et al., 2012). Pregnant women eligible for inclusion (n = 2,644) (i.e., at least 16 years of age, no communication difficulties, singleton pregnancy, no assisted fertility programme, intention to give birth at the reference hospital) were recruited at their first pre-natal visit (10-13 weeks of gestation) in the main public hospital or health center of each study area. Recruitment was done between November 2003 and February 2008. After excluding the women who withdrew from the study, were lost to follow-up, had induced or spontaneous abortions, or had fetal deaths, a sample of 2,034 children were followed-up to 5 years of age. Final analysis included 1,516 (74.5%) children with teacher-rated ADHD symptoms questionnaire based on the diagnostic criteria for ADHD as described in the Diagnostic and Statistical Manual of Mental Disorders, fourth Edition (ADHD-DSM-IV) (American Psychiatric Association, 2000) (**Figure 1**). **Table 1** shows the characteristics of the included (n = 1,516) and excluded (n = 1,128) population. The included participants had higher parental educational level and social class, older parents, a higher proportion were breastfed for longer, presented lower in utero tobacco exposure and higher cord blood mercury concentrations compared to the excluded population.

Approval was given by the ethical committees of the centers involved in the study, and the parents of all children provided signed informed consent.

Exposure measurement: A total of 23 socio-environmental factors were analysed in this study and they are described in detail in **Table S1**. Briefly, the information was collected from a variety of sources: ad hoc interviewer-administered questionnaires by trained INMA personnel, clinical data, physical examinations, biological samples (urine, blood), diet determinants and environmental measurements. The following factors obtained from first trimester questionnaire were explored in the study analysis: parental age, parental education, parental social class, parental country of birth, maternal pre-pregnancy body mass index, parity, and marital status. The following factors were included from the third trimester questionnaire: folic acid supplementation, seafood consumption, weight gain during pregnancy, maternal smoking, alcohol and paracetamol use, maternal environmental tobacco smoke exposure, and maternal mental health. Breastfeeding was assessed when children were six and 14 months old. The following covariates were included from the follow-up at age 4-5 years: a proxy of maternal verbal IQ, parental mental health, use of gas appliances at home, and child environmental tobacco smoke exposure. Several biomarkers were measured including maternal vitamin D concentrations, maternal and child cotinine levels, and mercury concentrations from cord blood. Information related to the child's birth, including date, gestational age, gender, and birth anthropometry were available from clinical records. Finally, data on outdoor air pollution (NO_2, benzene) throughout the pregnancy was obtained using LUR models.

Assessment of attention deficit hyperactivity disorder-like symptoms at age 5 years: ADHD symptoms were reported by teachers filling out the ADHD Criteria of *Diagnostic and Statistical Manual of Mental Disorders*, fourth Edition (ADHD-DSM-IV) list (American Psychiatric Association, 2000). ADHD-DSM-IV consists of 18 symptoms categorized in two separate symptom groups (inattention and hyperactivity/impulsivity) with nine symptoms each. Each ADHD symptom is rated on a 4-point scale of frequency from never or rarely (0) to very often (3), and subscales

can therefore be scored from 0 to 27, and the global score can range from 0 to 54. Higher scores indicate higher symptomatology.

Statistical analysis: Only subjects with complete data on ADHD-DSM-IV list, age and gender were included. Among these subjects, multiple imputation of missing values for the remaining variables was performed using chained equations where M=10 completed datasets were generated.

We have employed the deletion/substitution/addition algorithm (DSA) (Sinisi & van der Laan, 2004) on multiple imputed data when selecting a model for ADHD, Inattention and Hyperactivity/Impulsivity scores, in all children and by gender.

DSA is an aggressive model search algorithm based on cross-validation (CV) and the L2 loss function. The set of candidate models is limited by three user-defined parameters: the maximum model size (i.e., the number of terms that a model contains), the maximum order of interaction amongst predictors, and the maximum power for a given predictor.

Starting from a model with only an intercept, the DSA algorithm searches the optimal model for each size by making different moves. At each step, it chooses between removing a term (deletion move), replacing one term with another (substitution move), and adding a term to the current model (addition move). The acceptation of moves is based on the empirical loss function.

The final model is then selected based on V-fold CV among the optimal models for each possible model size. With this method, data are partitioned into v training datasets. At each of v-times, the data are trained on v-1 partitions and cross-validated on the left out dataset. The final model minimizes the CV risk average, i.e., the average loss function over the validation datasets.

We allowed no polynomial or interaction terms, and we considered models including up to 15 covariates. We tested models using 5-fold cross-validation (CV), i.e., each candidate model was fit and cross-validated five times at every step in the model building.

The final model was applied to each imputed dataset, and their estimates then combined into one overall estimate and variance, incorporating both the within and between imputation variability. Zero-inflated negative binomial (ZINB) mixed effects models (including INMA sub-cohort as random effect) were used to address the multilevel nature of the data and account for over-dispersion and overrepresentation of zero values of the outcome (Vilor-Tejedor et

al., 2016). The results are presented as Rate Ratios (RR) with corresponding 95% confidence intervals (95% CI).
Statistical analyses were performed using R (version 3.2.5; R Foundation for Statistical Computing) implementations of the statistical packages DSA and glmmADMB.

RESULTS

Overall, 51% of the population sample were boys, 28% were born in the last quartile of the year (from October to December), 5% were preterm, mothers had a mean age of 31 years, and 37% had a university degree (**Table 1**). A total of 1,215 (80%) children had an ADHD symptoms score of 1 or more (mean = 8.8; SD = 8.3). More boys (54%) were rated with ADHD symptoms compared to girls and they presented higher scores (mean = 10.4; SD = 9.4; mean = 6.9; SD = 6.4, respectively).
Figure 2 shows the average CV risk of the candidate models chosen by the DSA algorithm as a function of the size of the model. The crude estimates are shown in **Table S2**. Models selected using the DSA algorithm are outlined in **Table 2** in which we present the associations between exposure to socio-environmental factors and ADHD symptoms score at 5 years of age. We found male gender (RR = 1.67, 95% CI 1.50–1.87), younger relative age (Q4 vs Q1: RR = 1.58, 95% CI 1.35–1.85), low social class (RR = 1.18, 95% CI 1.05–1.33), maternal depression (RR = 1.20, 95% CI 1.00–1.45), paternal hostility (RR = 1.21, 95% CI 1.05–1.41), environmental tobacco smoke (RR = 1.04, 95% CI 1.02–1.07), and prenatal alcohol consumption (RR = 1.04, 95% CI 1.01–1.07) to be associated with higher ADHD symptoms score in a birth cohort of preschool children.
DSA algorithm selected postnatal rather than prenatal tobacco exposure, although the association between the two exposure periods is high (90% of children with the highest exposure levels during pregnancy showed high levels of cotinine at 4-5 years follow-up) (**Table S3**).
Stratifying results by ADHD symptom domains, inattention symptoms score showed more associations with socio-environmental factors compared to hyperactive/impulsive symptoms score (**Table 2**).
Stratifying results by gender (**Table 3**), psychosocial factors such as low social class, worse parental mental health (i.e., higher SCL-90R

raw scores) and maternal obesity showed an association with ADHD symptoms score in boys, and prenatal exposure to toxics (i.e., smoking and alcohol) was associated with ADHD symptoms score in girls.

DISCUSSION

This large population-based study (n=1,516) followed a naïve analysis for examining numerous socio-environmental factors associated with the development of preschool symptoms of ADHD using a novel statistical method to deal with correlated data. We found male gender, younger relative age, low social class, worse parental mental health, pre-pregnancy obesity, environmental tobacco smoke and prenatal alcohol consumption to be associated with ADHD symptoms score in a birth cohort of preschool children. However, we did not find significant associations with other frequently reported risk factors such as prematurity or low birth weight. Finally, ADHD symptom domains and gender differences in susceptibility to socio-environmental factors were encountered.

According to our results, gender was the factor that showed the strongest association with ADHD symptoms score, males being the more vulnerable. Previous literature consistently showed for boys higher rates of many neurodevelopmental disorders such as ADHD, autism, antisocial behaviour, developmental language disorders or dyslexia (Thibaut, 2016). Causes for gender differences include genetic influences, hormonal effects, brain structure/function (including maturation and vulnerability) and psychosocial influences (Rutter, Caspi, & Moffitt, 2003). Regarding date of birth, younger relative age within the school year associated with an increased likelihood of an ADHD diagnosis has been reported previously in many independent groups in different countries (Chen et al., 2016; Hoshen, Benis, Keyes, & Zoëga, 2016; Librero, Izquierdo-María, García-Gil, & Peiró, 2015; Morrow et al., 2012; Sayal, Chudal, Hinkka-Yli-Salomäki, Joelsson, & Sourander, 2017). In our study, we also found that children born in the last quarter of the year (the youngest group in the class) were 58% more likely to be rated with ADHD symptoms compared to the oldest children. A biological explanation seems unlikely (e.g., season birth effect) since this significant difference in risk may occur within a span of only a few days (Ford-Jones, 2015) but it suggests the informants

difficulties for observing ADHD-related symptoms independent of the relative maturity of children, particularly in boys.

We found exposure to environmental tobacco smoke and maternal alcohol intake during pregnancy as risk factors associated with ADHD symptoms in line with previous research (Han et al., 2015; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002), even though the nature of the association is still under debate (Huang et al., 2018; Thapar & Rutter, 2009). Due to highly associated data, the selection of postnatal and not prenatal tobacco exposure as a risk factor may be based on the worst effects of a long term exposure. Alcohol is a potent teratogen associated with the development of fetal alcohol spectrum disorders (FASD) which include irreversible physical, behavioural and cognitive problems, such as ADHD. However, not all the babies exposed to alcohol will develop FASD and literature is inconsistent with regard to lower levels of alcohol use in pregnancy (Thapar et al., 2013). In our cohorts, we did find a positive association with ADHD symptoms score. We think this is a relevant finding, and a more than likely potential underestimation of the effect taking into account that the exposure measurement was based on maternal recall and self-misreporting cannot be discarded. In fact, a recent study using objective measures found alarming misreported prevalence of alcohol intake during gestation (although only 3% of mothers stated to have consumed alcohol during pregnancy, hair biomarkers showed that only 35% had been totally abstinent) (Gomez-Roig et al., 2017). Previously reported biological mechanisms in animal and human studies provide support for the adverse effects encountered of smoking and alcohol exposure in neurodevelopment (Patten, Fontaine, & Christie, 2014; Shea & Steiner, 2008; Terasaki & Schwarz, 2016).

For psychosocial factors, we found that ADHD symptoms score increased in lower social classes and among children with worse parental psychopathology (Biederman et al., 2002; Joelsson et al., 2017). It was back in the 1970s when Rutter and colleagues firstly explored the association between psychosocial adversity within the family environment and childhood mental disorders and finding positive correlations (M. Rutter et al., 1975; M. Rutter, Tizard, Yule, Graham, & Whitmore, 1976).

In stratified analysis, we found that for boys, psychosocial factors were associated with ADHD symptoms score and by contrast, prenatal exposure to drugs (i.e., smoking and alcohol) were associated with ADHD symptoms score in girls. Previous literature

describes no differences by gender in the associations between ADHD symptoms and adversity factors (Biederman et al., 2002; Silva et al., 2014), although a study carried out by Sayal et al. (2007) did also find female-specific associations with very low levels of alcohol consumption during pregnancy and childhood mental health. However, animal studies seem not to support a female-specific effect of prenatal alcohol exposure (Terasaki & Schwarz, 2016) and additional research is needed to explicate the meaning of these findings. Nevertheless, the outcome measure (teacher-reported symptoms) implications should be considered (e.g., a hypothesized higher sensitivity for ADHD symptomatology in the females subsample enhancing risk detection of tobacco and alcohol exposure during the fetal period on neurodevelopment). This explanation would go beyond the psychometric properties of the ADHD-DSM-IV form list but we raise it in the context of a societal male-gender bias to symptoms detection/interpretation, identification of dysfunction, and clinical or resource referral of boys compared to girls not only justified by higher rates of comorbidities (Biederman et al., 2005). Regarding ADHD symptoms domains, we found a much larger number of socio-environmental risks associated with inattention symptoms score. These findings were unexpected since in Willcutt et al. (2010) low genetic influence for the hyperactive/impulsive type is suggested, and therefore a higher role for environmental factors.

Finally, we did not find associations with other frequently reported risk factors such as birth weight or gestational age (Halmøy, Klungsøyr, Skjærven, & Haavik, 2012; Mick, Biederman, Prince, Fischer, & Faraone, 2002; Serati, Barkin, Orsenigo, Altamura, & Buoli, 2017). However, children with low birth weight represent a small proportion of children with ADHD (Mick, Biederman, Prince, et al., 2002). Therefore, the null findings of other previously reported risk factors could be related to, firstly a misclassification of the outcome in children aged 4-5 years. Secondly, a lack of statistical power due a non-differential exposure in children with higher ADHD symptoms score compared to children with low or zero ADHD symptoms score. Thirdly, lack of independence or collinearity between exposures. Finally, the most determinant factors in a population-based sample are the ones selected.

Our study faced some limitations that need to be considered. Although we found an association between several environmental factors and ADHD score, it is not possible to disentangle

environmental influences from genetic effects with our research design (Biederman et al., 2002). Furthermore, it is difficult to separate prenatal from postnatal effects (i.e., socio-economic characteristics) (Sciberras et al., 2017). Although despite uncertainties in relation to critical windows of exposure, the identification of key socio-environmental risks is still valid. Data on the 18 ADHD symptoms in DSM-IV were collected by teacher ratings and are not to be confounded with a medical diagnosis of ADHD. However, behaviour rating scales for assessing ADHD symptoms have shown moderate to high sensitivity and specificity in detecting ADHD in children and adolescents (Chang, Wang, & Tsai, 2016). Attention assessment would have benefited from a multi-informant approach (e.g., teachers and parents), although the validity of teacher ratings, especially in relation to externalizing behaviours and prediction of long-term impairment, has been reported previously (Hinshaw, Han, Erhardt, & Huber, 1992; Mannuzza, Klein, & Moulton, 2002). The study of the early precursors associated with ADHD in preschoolers is indeed an area where further research is needed (Döpfner, Rothenberger, & Sonuga-Barke, 2004; Egger & Angold, 2006) although differentiating developmentally healthy levels of symptoms from clinically significant behaviours in very young children can be difficult (Döpfner et al., 2004). Nevertheless, the low prevalence rates of ADHD symptoms in preschoolers suggest that the DSM-IV ADHD criteria are not descriptive of a typical child behaviour (Egger & Angold, 2006). Furthermore, prospective longitudinal studies have encountered persistency of ADHD symptoms in preschoolers through elementary school and into adolescence (Spira & Fischel, 2005). Finally, another limitation is reliance on self-report for some exposure measurements which can be imprecise.

Strengths of this population-based study include the large prospective non-clinical birth cohort. However, extrapolation of the results to the general population requires caution given the socio-demographic characteristics of our study population. Another strength is the large number of socio-environmental factors analysed, including psychosocial and physical factors. Furthermore, the use of the outcome as a continuous measure enabled dose-response evaluations and greater statistical power in the analysis. Finally, differences by gender and ADHD symptom domains could also be explored.

Conclusions

In our study population, among a large set of socio-environmental factors, only male gender, younger relative age, psychosocial adversity, smoking and prenatal exposure to alcohol were associated with ADHD symptoms at preschool age, pointing towards these would be the most relevant determinants.
ADHD symptoms exist in preschoolers, and they are at increased risk for emotional, behavioural, academic and social difficulties. Furthering research on ADHD development is needed in order to reduce its psychosocial impact.

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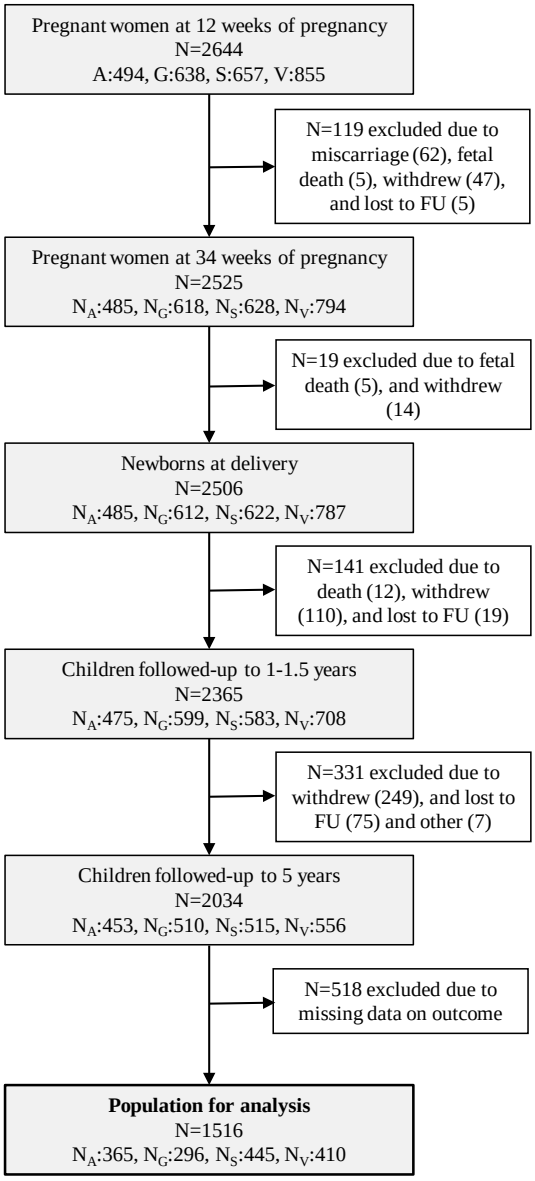

N: Number of observations, N_X : Number of observations by sub-cohort, where X = A: Asturias, G: Gipuzkoa, S: Sabadell, and V: Valencia; FU: follow-up
† Not available for Valencia sub-cohort

Figure 1. Study method flowchart.

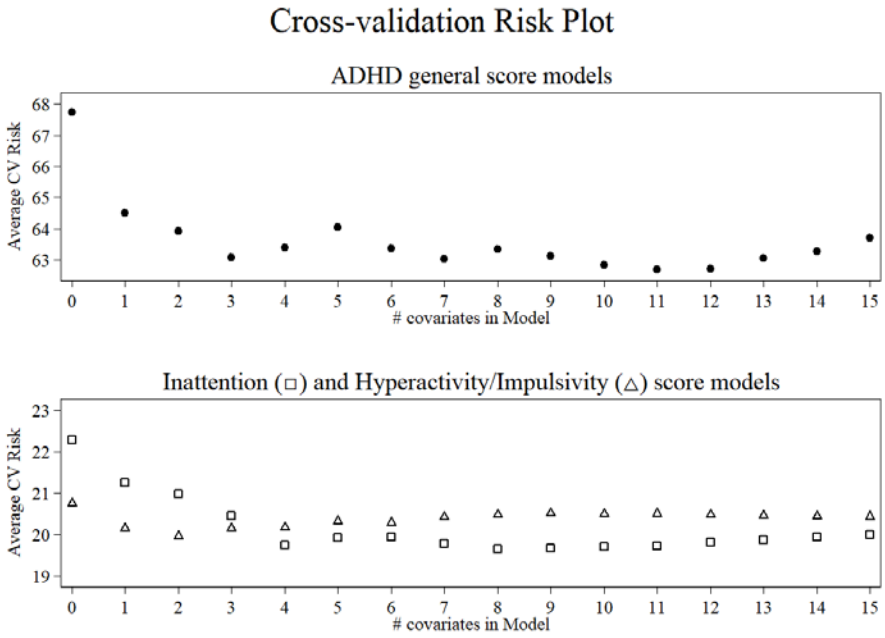

Figure 2. CV risk plotted against size of the models selected by DSA algorithm.

Table 1. Description of the socio-environmental risk factors analyzed stratified by included and excluded population.

	Included (N=1516)		Excluded (N=1128)		
	n	% or Mean (SD)	n	% or Mean (SD)	p-value*
PRENATAL RISK/PROTECTIVE FACTORS					
Folic acid, mg/day	1516	964.9 (1394.7)	1091	896.5 (1380.4)	0.049
Vitamin D, ng/mL	1446	29.9 (11.4)	1043	30.5 (11.9)	0.367
Seafood, gr/week	1503	508.0 (297.3)	861	486.2 (273.6)	0.247
Smoking history (pre-natal)					
Maternal urine cotinine concentration					<0.001
< 4 ng/mL (=LOD)	594	42.5%	305	34.2%	
≥ 4 ng/mL	804	624.0 (1181.8)	586	736.3 (1338.3)	
Smoking during pregnancy, yes	430	29.0%	350	36.1%	<0.001
Second hand smoke during pregnancy, yes	874	59.1%	654	67.8%	<0.001
Alcohol, gr/day	1516	3.9 (16.4)	1090	4.3 (15.4)	0.145
Paracetamol, ever	674	45.9%	461	43.7%	0.164
Date of birth, Q4	424	28.0%	258	26.1%	0.724
Gender, male	777	51.3%	513	52.0%	0.774
Birth weight, gr	1514	3258.4 (463.4)	974	3245.9 (511.7)	0.273
Low birth weight, yes	72	4.8%	56	5.7%	0.973
Gestational age, weeks	1516	39.6 (1.6)	985	39.5 (1.9)	0.710
Preterm, yes	69	4.6%	48	4.9%	0.270
Weight gain during pregnancy					0.087
Recommended	580	39.5%	343	36.3%	
Low	342	23.3%	238	25.2%	
High	548	37.3%	364	38.5%	
POSTNATAL RISK/PROTECTIVE FACTORS					
Breastfeeding, weeks					<0.001
0	220	14.8%	122	14.8%	
>0-16	349	23.5%	237	28.7%	
>16-24	251	16.9%	114	13.8%	
>24	664	44.7%	353	42.7%	
PSYCHOSOCIAL FACTORS					
Maternal age, years	1515	31.1 (4.0)	1119	29.9 (4.8)	<0.001
Paternal age, years	1513	33.1 (4.8)	1086	32.2 (5.4)	<0.001
Maternal social class					<0.001
High	354	23.4%	202	18.5%	
Medium	419	27.7%	251	23.0%	
Low	742	49.0%	638	58.5%	
Paternal social class					<0.001
High	319	21.6%	181	17.0%	
Medium	274	18.5%	161	15.2%	
Low	887	59.9%	720	67.8%	

Maternal education					<0.001
Primary or less	303	20.0%	348	32.0%	
Secondary	649	42.9%	429	39.4%	
University	560	37.0%	312	28.7%	
Paternal education					0.016
Primary or less	499	33.1%	431	39.8%	
Secondary	669	44.4%	454	41.9%	
University	340	22.5%	199	18.4%	
Parity					
0	874	57.7%	590	54.2%	0.002
1	552	36.5%	406	37.3%	
2+	88	5.8%	93	8.5%	
Marital status, stable partner	1499	98.9%	1061	97.3%	0.757
Maternal mental health, raw score	1365	0.7 (0.5)	-		
Paternal mental health, raw score	1182	0.6 (0.4)	-		
Maternal verbal IQ, raw score	1401	16.3 (4.0)	-		
Maternal ethnic group, not European	58	3.8%	104	9.6%	<0.001
Paternal ethnic group, not European	60	4.0%	131	12.0%	0.653
BMI (pre-pregnancy)					
Normal	1107	73.0%	804	73.9%	
Overweight	284	18.7%	205	18.8%	
Obese	125	8.2%	79	7.3%	
ENVIRONMENTAL TOXINS					
Environmental tobacco smoke (postnatal)					
Child's urine cotinine concentration					
< 4 ng/mL (=LOD)	715	55.1%	-		
≥ 4 ng/mL	582	32.4 (52.9)	-		
Second hand smoke, yes	408	27.8%	-		
Residential indoor air pollution, use of gas appliances at home, yes					
Prenatal	647	43.5%	439	45.3%	0.402
Postnatal	473	32.2%	-		
Residential outdoor air pollution, pre-natal					
NO_2 ($\mu g/m^3$)	1477	27.4 (11.7)	965	28.0 (12.2)	0.465
Benzene ($\mu g/m^3$)	1450	1.5 (0.9)	961	1.5 (0.9)	0.292
Mercury (pre-natal)					<0.001
< 2 μg/L (=LOD)	44	3.8%	45	6.1%	
≥ 2 μg/L	1105	12.0 (9.0)	692	10.5 (8.7)	

LOD: Limit Of Detection; SD: Standard Deviation; Q4: Quartile 4, Children born from October 1 to December 31; IQ: Intelligence Quotient; BMI: Body Mass Index; NO_2: Nitrogen dioxide.

- assessed at age 4-5 follow-up

* Mann-Whitney test for continuous variables; Values < LOD were replaced by sqrt(LOD). Chi-Square test for categorical variables.

Table 2. Socio-environmental factors associated to ADHD-DSM-IV outcomes.

	ADHD general score			Inattention			Hyperactivity/Impulsivity		
	RR (95% CI)	**p-value**	**FMI**	**RR (95% CI)**	**p-value**	**FMI**	**RR (95% CI)**	**p-value**	**FMI**
Pre- and postnatal risk/protective factors									
Gender, male	1.67 (1.50, 1.87)	<0.001	0.012	1.81 (1.59, 2.05)	<0.001	0.011	1.58 (1.38, 1.81)	<0.001	0.002
Date of birth, month = June	1.20 (0.94, 1.52)	0.135	0.010						
Date of birth, Q4	1.58 (1.35, 1.85)	<0.001	0.002	2.26 (1.87, 2.73)	<0.001	0.006			
Date of birth, Q3	1.52 (1.29, 1.79)	<0.001	0.006	2.07 (1.71, 2.51)	<0.001	0.009			
Date of birth, Q2	1.24 (1.03, 1.49)	0.024	0.009	1.52 (1.25, 1.84)	<0.001	0.014			
Pre-natal alcohol consumption, per 10 gr increase	1.04 (1.01, 1.07)	0.023	0.185	1.05 (1.01, 1.09)	0.014	0.243			
Duration of breastfeeding, >24 weeks	0.89 (0.80, 1.01)	0.061	0.052				0.80 (0.70, 0.93)	0.002	0.046
Environmental toxins									
Child's urine cotinine conc. at age 4 years, log transformed	1.04 (1.02, 1.07)	0.003	0.171	1.06 (1.02, 1.10)	<0.001	0.239			
Psychosocial factors									
Maternal social class, IV+V	1.18 (1.05, 1.33)	0.004	0.030	1.31 (1.15, 1.49)	<0.001	0.028			
Maternal diagnosed depression	1.20 (1.00, 1.45)	0.052	0.009						
Paternal mental health, hostility (SCL-90R)	1.21 (1.05, 1.41)	0.011	0.317	1.28 (1.10, 1.48)	0.001	0.128			

RR: Rate Ratio; CI: Confidence Interval; FMI: Fraction Missing Information, proportion of the total sampling variance that is due to missing data; Date of birth: Q2: 2nd quartile: children born from April 1 to June 30; Q3: 3rd quartile: children born from July 1 to September 30; Q4: 4th quartile: children born from October 1 to December 31; SCL-90R: Symptom Checklist-90-R.

Table 3. Socio-environmental factors associated to ADHD-DSM-IV outcomes: results stratifed by gender.

	ADHD general score			Inattention			Hyperactivity/Impulsivity		
	RR (95% CI)	**p-value**	**FMI**	**RR (95% CI)**	**p-value**	**FMI**	**RR (95% CI)**	**p-value**	**FMI**
MALES									
Pre- and postnatal risk/protective factors									
Date of birth, season = winter	0.76 (0.64, 0.90)	0.002	0.007						
Date of birth, Q2				1.72 (1.35, 2.20)	<0.001	0.013			
Date of birth, Q3				1.88 (1.48, 2.39)	<0.001	0.009			
Date of birth, Q4				2.12 (1.67, 2.69)	<0.001	0.010			
Post-natal risks									
Duration of breastfeeding, >24 weeks	0.88 (0.75, 1.03)	0.117	0.057				0.78 (0.64, 0.94)	0.009	0.056
Environmental toxins									
Child's urine cotinine conc. at age 4 years, log transformed	1.06 (1.02, 1.10)	0.007	0.232	1.07 (1.02, 1.11)	0.004	0.182			
Psychosocial factors									
Maternal social class, IV+V	1.22 (1.04, 1.43)	0.014	0.063	1.23 (1.04, 1.45)	0.016	0.041			
Paternal mental health, hostility (SCL-90R)	1.32 (1.10, 1.57)	0.003	0.148	1.30 (1.07, 1.58)	0.008	0.137			
Maternal mental health, Paranoid ideation (SCL-90R)				1.12 (0.99, 1.28)	0.077	0.048			
Maternal pre-pregnancy BMI, obese				1.35 (1.04, 1.75)	0.024	0.014			
FEMALES									
Pre- and postnatal risk/protective factors									
Pre-natal urine cotinine conc., log-transformed							1.05 (1.01, 1.09)	0.017	0.387
Pre-natal alcohol consumption, per 10 gr increase	1.04 (1.00, 1.08)	0.066	0.003						
Date of birth, Q3	1.52 (1.23, 1.89)	<0.001	0.003	2.21 (1.71, 2.86)	<0.001	0.003			
Date of birth, Q4	1.46 (1.18, 1.79)	<0.001	0.003	2.34 (1.82, 3.02)	<0.001	0.003			

RR: Incidence Rate Ratio; CI: Confidence Interval; FMI: Fraction Missing Information, proportion of the total sampling variance that is due to missing data; Date of birth: Q2: 2nd quartile: children born from April 1 to June 30; Q3: 3rd quartile: children born from July 1 to September 30; Q4: 4th quartile: children born from October 1 to December 31; SCL-90R: Symptom Checklist-90-R; BMI: Body Mass Index.

Supplementary material

Table S1. Description of the socio-environmental factors analysed.

	EXPOSURE				
SOCIO-ENVIRONMENTAL FACTORS	**MEASUREMENT**	**METHOD**	**MEASUREMENT UNITS**	**TIME POINT ASSESSMENT**	**References**
PRE- AND POSTNATAL RISK/PROTECTIVE FACTORS					
Folic acid	Doses of folic acid (supplement)	Semi quantitative food frequency questionnaire	mg/day	From one month before conception to the seventh month of pregnancy	(Valera-Gran et al., 2014)
Vitamin D	Maternal plasma concentrations of 25(OH)D3	High-performance liquid chromatography method using BioRAD kit	ng/mL	First trimester of pregnancy (10-13 weeks)	(Morales et al., 2015)
Seafood	Seafood subtypes: large fatty fish, small fatty fish, lean fish, shellfish, smoked/dried/salted fish	Semi-quantitative food frequency questionnaire	gr/week	Two periods: First period from 3 months before conception to the third month of pregnancy. Second period from fourth to seventh month of pregnancy	(Julvez et al., 2016)
Smoking history	Maternal urine cotinine concentration	Competitive enzyme immunoassay in urine samples	ng/mL	Third trimester of pregnancy (28-32 weeks)	(Morales et al., 2016)
	Active smoking during pregnancy	Interviewer-administered questionnaire	Yes/no	Third trimester of pregnancy	(Julvez et al., 2007)

	Second hand smoke during pregnancy	Interviewer-administered questionnaire	Yes/no (in several environments: home, workplace, in restaurants and leisure areas such as pubs or bars)	Third trimester of pregnancy	(Iñiguez et al., 2012)
Alcohol	Wine, beer, white and brown spirits, liqueurs	Semi quantitative food frequency questionnaire	gr/day	First and third trimester of pregnancy	
Paracetamol	Paracetamol doses	Interviewer-administered questionnaire	Never, sporadic, persistent	First and third trimesters of pregnancy	(Avella-Garcia et al., 2016)
Date of birth	Relative age	Clinical records	Quartiles	At birth	(Kowalyk, Davis, Wattie, & Baker, 2014)
Gender	Gender	Clinical records	Male/female	At birth	
Birth weight	Weight	Clinical records	gr Normal, low (<2.500 gr)	At birth	
Gestational age	Weeks of gestation	Clinical records	Weeks Term, preterm (<37 weeks)	At birth	
Fetal growth	Fetal growth restriction for weight, length, head circumference	Customised model (constitutional characteristics)	Yes/no	At birth	(Chatzi et al., 2012)
Breastfeeding	Duration of any breastfeeding	Interviewer-administered questionnaire	0 weeks, >0-16 weeks, >16-24 weeks, >24 weeks	At six months (Asturias and Sabadell sub cohorts) and 14 months (all sub cohorts)	(Boucher et al., 2017)

PSYCHOSOCIAL FACTORS					
Parental age	Age	Interviewer-administered questionnaire	Years	First trimester of pregnancy	
Social class	Occupational social class	Interviewer-administered questionnaire	High, medium, low	First and third trimesters of pregnancy	(Freire et al., 2011)
Parental education	Highest educational level	Interviewer-administered questionnaire	Primary or less, primary, secondary	First trimester of pregnancy	
Parity	Parity was defined as the number of previous pregnancies that lasted at least 22 weeks	Interviewer-administered questionnaire	Primiparous, multiparous	First trimester of pregnancy	
Marital status	Stable partner	Interviewer-administered questionnaire	Yes/no	First trimester of pregnancy	
Maternal mental health	Diagnosed anxiety and/or depression	Interviewer-administered questionnaire	Yes/no	First trimester of pregnancy	
Parental mental health	Global severity index, positive symptom distress index, positive symptom total, somatisation, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation, psychoticism	SCL-90-R (self-reported questionnaire)	Points, raw score	Age 4-5 follow-up	

Maternal verbal IQ	Similarities subtest	WAIS III scale (interviewer-administered test)	Points, raw score	Age 4-5 follow-up	(Avella-Garcia et al., 2016)
Parents ethnic group	Ethnicity	Interviewer-administered questionnaire	European, not European	First trimester of pregnancy	
BMI (pre-pregnancy)	BMI	Self-reported pre-pregnancy weight and measured height	Normal, overweight, obese	First trimester of pregnancy	(Casas et al., 2013)
Weight gain during pregnancy	Classification according to US Institute of Medicine guidelines	Clinical records	Recommended, low, high	During pregnancy	(Vizcaino, Grimalt, Glomstad, Fernández-Somoano, & Tardón, 2014)
ENVIRONMENTAL TOXINS					
Environmental tobacco smoke	Urine cotinine concentration	Competitive enzyme immunoassay in urine samples	ng/mL	Age 4-5 follow-up	(Aurrekoetxea et al., 2016)
	Second hand smoke	Interviewer-administered questionnaire	Yes/no (when a household member reported smoking at home)	Age 4-5 follow-up	(Aurrekoetxea et al., 2016)
Residential indoor air pollution					
Pre-natal	Use of gas appliances at home	Interviewer-administered questionnaire	Yes (any type of gas cooker)/no (electric cooker and other)	Third trimester of pregnancy	(Vrijheid et al., 2012)

Post-natal	Use of gas appliances at home	Interviewer-administered questionnaire	Yes (any type of gas cooker)/no (electric cooker and other)	Age 1-1.5 follow-up Age 4-5 follow-up	
Residential outdoor air pollution					
Pre-natal	NO_2	Land-use regression models	µg/m3	Throughout the pregnancy (in a total of four sampling periods of one week each)	(Estarlich et al., 2011)
	Benzene	Land-use regression models	µg/m3	Throughout the pregnancy (in a total of four sampling periods of one week each)	(Estarlich et al., 2011)
Mercury					
Pre-natal	Cord blood	Venipuncture of cord vessels before the placenta was delivered	µg/L	At birth	(Llop et al., 2017)

SCL-90R: Symptom Checklist-90-R; IQ: Intelligence Quotient; WAIS III: Wechsler Adult Intelligence Scale III; BMI: Body mass index; NO_2: Nitrogen dioxide.

Table S2. Crude estimates.

Zero-inflated Negative Binomial models. Adjusted for age at assessment and gender as fixed effects, and cohort as random effect.

	ALL				Females				Males			
	N obs.	RR	95% C.I.	p-value	N obs.	RR	95% C.I.	p-value	N obs.	RR	95% C.I.	p-value
PRE- AND POSTNATAL RISK/PROTECTIVE FACTORS												
Folic acid supplementation, mcg/day, per Δ500	1516	0.99	0.97, 1.01	0.379	739	1.00	0.97, 1.04	0.807	777	0.98	0.95, 1.01	0.184
12-weeks plasma vitamin D levels, log- ng/mL, per Δ1	1446	0.93	0.89, 0.99	0.014	710	0.95	0.87, 1.02	0.168	736	0.92	0.85, 0.99	0.029
TSH levels, log- µU/mL, per Δ1	1448	0.94	0.87, 1.01	0.073	701	0.89	0.80, 0.99	0.030	747	0.99	0.89, 1.10	0.904
T4 levels, L/pmol, per Δ0.1	1448	1.01	0.65, 1.56	0.973	701	1.47	0.73, 2.94	0.279	747	0.83	0.48, 1.43	0.497
Seafood intake during pregnancy, per 10 adj gr/week	1503	0.83	0.55, 1.23	0.349	731	0.59	0.31, 1.10	0.098	772	1.05	0.63, 1.75	0.841
Maternal 32-weeks urine cotinine levels (ng/mL), ref. < 50												
50 -2000	1398	1.26	1.06, 1.50	0.008	673	1.46	1.13, 1.88	0.004	725	1.12	0.89, 1.40	0.344
> 2000	1398	1.40	1.13, 1.74	0.002	673	1.32	0.94, 1.84	0.110	725	1.49	1.13, 1.98	0.005
Maternal smoking at any time during pregnancy, yes	1485	1.22	1.08, 1.39	0.002	721	1.29	1.07, 1.55	0.007	764	1.17	0.99, 1.39	0.061
Second hand smoke during pregnancy, yes	1479	1.05	0.94, 1.19	0.385	718	0.98	0.82, 1.18	0.856	761	1.11	0.95, 1.30	0.205
Maternal alcohol intake during pregnancy, gr/day, per Δ10	1516	1.00	1.00, 1.01	0.041	739	1.00	1.00, 1.01	0.040	777	1.00	1.00, 1.01	0.506
Maternal exposure to paracetamol during pregnancy, Ever vs Never	1468	1.06	0.94, 1.20	0.331	713	1.14	0.94, 1.37	0.179	755	1.02	0.87, 1.20	0.802
Child's date of birth, relative age, ref. Q1												
Q2	1516	1.36	1.15, 1.60	<0.001	739	1.18	0.91, 1.53	0.209	777	1.51	1.21, 1.88	<0.001
Q3	1516	1.59	1.35, 1.88	<0.001	739	1.69	1.31, 2.18	<0.001	777	1.53	1.23, 1.89	<0.001
Q4	1516	1.62	1.38, 1.90	<0.001	739	1.64	1.28, 2.10	<0.001	777	1.58	1.28, 1.96	<0.001
Child's date of birth, season, ref. Spring												
Summer	1516	1.20	1.02, 1.41	0.028	739	1.37	1.07, 1.76	0.014	777	1.09	0.88, 1.35	0.421
Autumn	1516	1.21	1.03, 1.42	0.022	739	1.35	1.05, 1.74	0.020	777	1.10	0.89, 1.36	0.365
Winter	1516	0.87	0.74, 1.02	0.094	739	1.00	0.78, 1.29	1.000	777	0.78	0.63, 0.97	0.026

Child's birth weight, per Δ100 gr	1514	1.00	0.98,	1.01	0.525	739	1.00	0.98,	1.02	0.981	775	0.99	0.98,	1.01	0.314
Low birth weight, yes	1514	1.06	0.82,	1.38	0.643	739	0.95	0.68,	1.34	0.775	775	1.24	0.82,	1.87	0.300
Gestational age, per Δ1 week	1516	1.01	0.97,	1.04	0.775	739	1.02	0.97,	1.08	0.367	777	0.99	0.94,	1.04	0.613
Prematurity, yes	1516	1.14	0.88,	1.48	0.334	739	1.06	0.71,	1.59	0.768	777	1.20	0.85,	1.70	0.295
Duration of breastfeeding, ref. no breastfed															
>0-16 weeks	1484	1.06	0.88,	1.28	0.510	726	1.03	0.79,	1.35	0.826	758	1.09	0.85,	1.41	0.503
>16-24 weeks	1484	0.99	0.81,	1.21	0.920	726	0.92	0.68,	1.25	0.611	758	1.03	0.79,	1.34	0.825
>24 weeks	1484	0.86	0.73,	1.02	0.090	726	0.85	0.66,	1.10	0.221	758	0.86	0.68,	1.08	0.185
PSYCHOSOCIAL FACTORS															
Maternal age, per Δ5 years	1515	0.94	0.88,	1.01	0.087	739	0.93	0.84,	1.03	0.142	776	0.95	0.87,	1.04	0.300
Paternal age, per Δ5 years	1513	0.99	0.94,	1.05	0.754	737	0.96	0.88,	1.05	0.356	776	1.01	0.94,	1.08	0.735
Maternal social class, ref. CS I+II															
CS III	1515	1.12	0.95,	1.31	0.165	739	1.13	0.89,	1.44	0.309	776	1.13	0.92,	1.39	0.251
CS IV+V	1515	1.37	1.18,	1.58	<0.001	739	1.34	1.08,	1.67	0.009	776	1.42	1.18,	1.71	<0.001
Paternal social class, ref. CS I+II															
CS III	1480	1.04	0.87,	1.24	0.704	724	1.20	0.91,	1.59	0.192	756	0.92	0.73,	1.16	0.466
CS IV+V	1480	1.20	1.04,	1.38	0.012	724	1.19	0.95,	1.48	0.124	756	1.23	1.02,	1.47	0.029
Maternal education, ref. Primary or less															
Secondary	1512	0.82	0.71,	0.95	0.009	739	0.83	0.66,	1.03	0.094	773	0.81	0.66,	0.98	0.034
University	1512	0.71	0.60,	0.82	<0.001	739	0.70	0.56,	0.88	0.003	773	0.70	0.57,	0.86	<0.001
Paternal education, ref. Primary or less															
Secondary	1508	0.84	0.74,	0.96	0.010	736	0.78	0.65,	0.95	0.012	772	0.90	0.76,	1.07	0.232
University	1508	0.82	0.70,	0.95	0.010	736	0.76	0.60,	0.97	0.025	772	0.86	0.70,	1.05	0.144
Birth order, ref. 0															
1	1514	1.01	0.90,	1.14	0.834	739	1.12	0.94,	1.34	0.216	775	0.93	0.79,	1.08	0.331
2+	1514	1.03	0.81,	1.31	0.817	739	1.04	0.73,	1.49	0.818	775	1.03	0.74,	1.44	0.857
Maternal diagnosed anxiety, yes	1514	1.06	0.90,	1.24	0.485	739	1.08	0.84,	1.38	0.558	775	1.06	0.86,	1.30	0.606
Maternal diagnosed depression, yes	1514	1.28	1.06,	1.54	0.010	739	1.30	0.99,	1.71	0.058	775	1.28	0.99,	1.65	0.060
Maternal mental health (SCL-90R), Global severity index	1365	1.13	1.01,	1.27	0.041	665	1.04	0.87,	1.24	0.669	700	1.21	1.04,	1.42	0.017

Maternal mental health (SCL-90R), Positive symptom distress index	1365	1.18	1.02,	1.35	0.025	665	1.09	0.88,	1.35	0.450	700	1.26	1.05,	1.52	0.014
Maternal mental health (SCL-90R), Positive symptom total	1365	1.00	1.00,	1.00	0.226	665	1.00	1.00,	1.00	0.940	700	1.00	1.00,	1.01	0.139
Maternal mental health (SCL-90R), Somatization	1365	1.09	0.99,	1.19	0.089	665	1.07	0.92,	1.23	0.374	700	1.10	0.97,	1.25	0.130
Maternal mental health (SCL-90R), Obsessive-compulsive	1365	1.03	0.94,	1.12	0.552	665	0.99	0.87,	1.12	0.820	700	1.07	0.95,	1.21	0.282
Maternal mental health (SCL-90R), Interpersonal sensitivity	1365	1.07	0.97,	1.17	0.157	665	1.03	0.89,	1.19	0.723	700	1.09	0.97,	1.23	0.158
Maternal mental health (SCL-90R), Depression	1365	1.09	1.00,	1.19	0.051	665	1.01	0.88,	1.16	0.865	700	1.16	1.03,	1.30	0.013
Maternal mental health (SCL-90R), Anxiety	1365	1.10	0.99,	1.21	0.065	665	1.04	0.90,	1.21	0.589	700	1.15	1.01,	1.32	0.038
Maternal mental health (SCL-90R), Hostility	1365	1.07	0.96,	1.19	0.245	665	0.98	0.82,	1.15	0.769	700	1.15	1.00,	1.33	0.056
Maternal mental health (SCL-90R), Phobic anxiety	1365	1.18	1.01,	1.37	0.032	665	1.18	0.94,	1.48	0.162	700	1.17	0.96,	1.43	0.112
Maternal mental health (SCL-90R), Paranoid ideation	1365	1.13	1.03,	1.24	0.011	665	1.00	0.86,	1.17	0.968	700	1.22	1.08,	1.38	0.001
Maternal mental health (SCL-90R), Psychoticism	1365	1.15	1.01,	1.31	0.037	665	1.05	0.87,	1.28	0.602	700	1.23	1.03,	1.46	0.019
Paternal mental health (SCL-90R), Global severity index	1182	1.24	1.06,	1.46	0.008	566	1.06	0.84,	1.34	0.620	616	1.42	1.14,	1.77	0.002
Paternal mental health (SCL-90R), Positive symptom distress index	1173	1.27	1.06,	1.51	0.009	560	1.08	0.83,	1.41	0.553	613	1.44	1.14,	1.82	0.002
Paternal mental health (SCL-90R), Positive symptom total	1182	1.00	1.00,	1.01	0.052	566	1.00	1.00,	1.01	0.875	616	1.01	1.00,	1.01	0.013
Paternal mental health (SCL-90R), Somatization	1182	1.19	1.04,	1.37	0.012	566	1.13	0.93,	1.38	0.230	616	1.25	1.03,	1.51	0.023
Paternal mental health (SCL-90R), Obsessive-compulsive	1182	1.17	1.04,	1.31	0.007	566	1.08	0.91,	1.27	0.369	616	1.24	1.06,	1.44	0.006
Paternal mental health (SCL-90R), Interpersonal sensitivity	1182	1.08	0.96,	1.22	0.201	566	0.93	0.78,	1.11	0.400	616	1.23	1.04,	1.46	0.013
Paternal mental health (SCL-90R), Depression	1182	1.18	1.03,	1.34	0.013	566	1.02	0.85,	1.24	0.803	616	1.31	1.10,	1.55	0.002
Paternal mental health (SCL-90R), Anxiety	1182	1.15	1.00,	1.33	0.053	566	1.03	0.83,	1.27	0.810	616	1.28	1.05,	1.55	0.013
Paternal mental health (SCL-90R), Hostility	1182	1.25	1.09,	1.44	0.002	566	1.15	0.94,	1.41	0.179	616	1.35	1.11,	1.63	0.002
Paternal mental health (SCL-90R), Phobic anxiety	1182	1.08	0.88,	1.33	0.452	566	1.11	0.83,	1.48	0.489	616	1.06	0.80,	1.40	0.695
Paternal mental health (SCL-90R), Paranoid ideation	1182	1.15	1.02,	1.31	0.021	566	1.03	0.86,	1.24	0.720	616	1.25	1.06,	1.48	0.009
Paternal mental health (SCL-90R), Psychoticism	1182	1.19	1.03,	1.38	0.021	566	1.05	0.85,	1.30	0.641	616	1.33	1.09,	1.63	0.006
Maternal IQ (Similarities subtest score; WAIS-III)	1401	0.99	0.97,	1.00	0.064	681	0.99	0.97,	1.01	0.250	720	0.98	0.97,	1.00	0.122
Maternal country of origin, not European	1515	1.07	0.80,	1.43	0.627	739	1.05	0.67,	1.64	0.844	776	1.11	0.76,	1.61	0.584
Paternal country of origin, not European	1513	1.02	0.77,	1.35	0.905	739	0.90	0.58,	1.39	0.627	774	1.13	0.78,	1.64	0.512
Mother's pre-pregnancy BMI, ref. Normal weight															
Overweight	1516	1.11	0.96,	1.28	0.157	739	1.10	0.89,	1.38	0.376	777	1.13	0.93,	1.36	0.207
Obese	1516	1.31	1.07,	1.60	0.010	739	1.17	0.84,	1.64	0.350	777	1.40	1.09,	1.81	0.009

IOM guidelines for pregnancy weight gain, Recommended															
Low	1470	0.97	0.84,	1.13	0.709	716	0.85	0.68,	1.07	0.163	754	1.12	0.92,	1.37	0.258
High	1470	1.07	0.94,	1.21	0.340	716	0.93	0.76,	1.13	0.458	754	1.22	1.02,	1.44	0.025
ENVIRONMENTAL TOXINS															
Child's urinary cotinine levels at age 4 years, ng/mL, ≥ 4 vs <4	1297	1.21	1.07,	1.36	0.002	612	1.28	1.07,	1.55	0.009	685	1.16	0.99,	1.35	0.070
Child's second hand smoke at age 4 years, yes	1467	1.15	1.02,	1.31	0.026	710	1.16	0.96,	1.40	0.126	757	1.15	0.97,	1.36	0.102
Type of cooker during pregnancy, any type of gas vs no gas cooker	1486	1.13	1.00,	1.28	0.053	721	1.08	0.90,	1.30	0.397	765	1.17	1.00,	1.39	0.056
Type of cooker at child's age 4 years, any type of gas vs no gas cooker	1470	1.11	0.97,	1.26	0.118	712	1.12	0.92,	1.35	0.267	758	1.10	0.93,	1.31	0.266
NO_2 levels during whole pregnancy ($\mu g/m^3$), per Δ10	1477	1.02	0.96,	1.09	0.501	718	1.03	0.94,	1.14	0.479	759	1.00	0.92,	1.09	0.954
Benzene levels during whole pregnancy ($\mu g/m^3$), per Δ1	1450	0.98	0.90,	1.07	0.695	701	0.94	0.82,	1.07	0.363	749	1.03	0.93,	1.14	0.579
Cord blood Hg levels, log-mcg/L, per Δ1	1149	0.99	0.91,	1.08	0.817	546	1.04	0.90,	1.20	0.596	603	0.96	0.86,	1.07	0.449

RR: Incidence Rate Ratio; TSH: Thyroid-stimulating hormone; Date of birth: Q2: 2nd quartile: children born from April 1 to June 30; Q3: 3rd quartile: children born from July 1 to September 30; Q4: 4th quartile: children born from October 1 to December 31; SCL-90R: Symptom Checklist-90-R; IQ: Intelligence quotient; BMI: Body Mass Index; IOM: Institute of Medicine.

Table S3. Association between smoking during pregnancy and environmental tobacco smoke.

	Maternal 32-weeks urine cotinine levels (ng/mL)		
	<50	50-2000	>2000
Child's urine cotinine levels at age 4-5 follow-up			
< LOD: 4 ng/mL, n (%)	608 (64.54)	43 (26.22)	9 (9.78)
≥ LOD: 4 ng/mL, n (%)	334 (35.46)	121 (73.78)	83 (90.22)
Min, Max	2, 346.7	3.1, 516.9	3.5, 359.9
Mean ± SD	22.5 (38.2)	41.6 (67.5)	57.6 (67.4)

LOD: Limit Of Detection; SD: Standard Deviation.

6. GENERAL DISCUSSION

6.1 Contributions and implications for public health

In the first study, we reviewed the latest research and sufficient evidence was encountered for an association between outdoor air pollution, particularly PAH, $PM_{2.5}$, and NO_x, and a negative impact on the neuropsychological development of children.

In the second study, we detected an ongoing development of some attention processes in primary school children. The developmental changes were more evident in executive attention and measures related to attentiveness, and in the younger groups. Furthermore, girls were more advantaged at younger ages and children with teacher-rated ADHD symptoms showed a delayed development in some attention processes.

The third study showed that daily levels of traffic-related ambient air pollution were associated with daily variations in attention processes in primary school children. Particularly, children showed higher inattentiveness on days with higher levels of ambient traffic-related air pollution.

In the fourth study, we found that prenatal, and at some extent postnatal, exposure to NO_2 at the residence address increased the risk of presenting signs of dyslexia in primary school children. This association remained after adjusting the models for age, gender, maternal education, residential neighborhood socioeconomic status, teacher-rated ADHD symptoms, birth weight, gestational age (prematurity), linguistic context at home, smoking during pregnancy, and outdoor school air pollution exposure.

In the fifth study, among a total of 23 socio-environmental factors related to ADHD, only male gender, younger relative age, psychosocial adversity, smoking and prenatal exposure to alcohol found to be associated with ADHD symptoms at preschool age using a novel method for handling correlated data.

In recent years evidence from human epidemiological and animal studies has been accumulating of the hazards of air pollution on the central nervous system. The review was an important contribution to updating previous works with the latest advances on the effects of outdoor air pollution and neuropsychological development in children.

The second study had implications for the measurement of cognitive development. The influence of different factors such as age, gender, indicators of socioeconomic status, or ADHD symptoms should be taken into account when studying cognitive development. We also provided a novel methodology in cognitive characterization by using repeated measurements within participants to define developmental trajectories. Furthermore, in addition to the three "standard" attention networks of the ANT, we calculated three more measures related to attentiveness which allowed us to explore the growth patterns of more aspects of attention. Finally, our study supported the child ANT as a valid and practical test for assessing attention processes in children in large epidemiological studies, particularly up to age 9 years.

The remaining three studies aimed to investigate the association between socio-environmental exposures and neurodevelopmental disorders. Urban pollution has been associated with short-term and long-term cardiovascular and respiratory effects (Kaiser, 2005; Mills et al., 2009; Peters et al., 2004; Seaton, MacNee, Donaldson, & Godden, 1995) although its acute cognitive effects, particularly in attention processes, had not yet been investigated and that was the novel contribution of the third study. Furthermore, the analysis of acute associations has the advantage that it is not sensitive to participants' individual or social characteristics (Sheppard et al., 2012). In the fourth study, we aimed to explore whether pre- and postnatal exposure to traffic-related air pollution was associated with warning signs of dyslexia in primary school children. To our knowledge, this was the first time to account for in utero exposure, and only one previous work investigated the associations with postnatal exposure to traffic air pollution (Fuertes et al., 2016). Our study, for the first time, pointed out to traffic air pollution as another environmental factor associated with signs of dyslexia, in addition to previously reported socioeconomic status or child health risk factors. Furthermore, these findings add to growing literature

that shows a detrimental effect of air pollution on brain maturation. In the fifth and last study, we followed a naïve approach that allowed us analyse a multiple set of factors using methods that handle correlated data. We proposed a novel modelling framework that addressed the issue of model selection when numerous variables jointly affect the outcome. We would like to emphasize the association encountered between maternal alcohol intake during pregnancy and ADHD symptoms. Alcohol is a well-known teratogen that can easily cross the placenta causing anomalies in the developing brain (Taléns-Visconti et al., 2011) and there is previous literature reporting its association with increased risk of ADHD (Han et al., 2015; Mick, Biederman, Faraone, Sayer, & Kleinman, 2002). Nevertheless, we still think it was a relevant finding in our cohorts taking into account that the exposure measurement was based on maternal recall through a semi quantitative food frequency questionnaire and therefore self-misreporting cannot be discarded. In fact, alarming misreported prevalence of alcohol intake during gestation have been previously reported (Gomez-Roig et al., 2017).

6.2 Strengths

The large sample size and the prospective and longitudinal designs of the two population-based studies included in this thesis. Furthermore, the use of nonreferred participants.

The direct measures of air pollution used, as well as the use of a standardized and validated air pollution assessment for estimating concentrations.

The inclusion of a large number of socio-environmental exposures that may be related to neurodevelopmental disorders, and the inclusion of numerous important covariates in the analyses.

The use of computerized tests in which efficiency, precision and objectivity of data collection is a major benefit in large-scale epidemiological studies.

The use of a specific and validated measure of ADHD symptoms; ADHD-DSM-IV is an internationally recognized form list (American Psychiatric Association, 2000). Furthermore, the dimensional perspective of ADHD enabled dose-response evaluations and greater statistical power in the analysis.

6.3 Limitations

A potential limitation is non-response or selection bias: Families that rejected being part of the study or children excluded because of incomplete data. In the first case, this non-response affects representativeness rather than internal validity. In the second case we used inverse probability weighting (IPW) so that results would be representative for the initial population.

Furthermore, another shortcoming of observational studies is the potential for residual confounding by socio-demographic characteristics; however, we adjusted our analyses for indicators of individual (i.e., maternal education) and residential neighborhood socioeconomic status which should have minimized such likelihood.

Air pollution levels were back-extrapolated to the pregnancy and early postnatal period, and this could lead to a nondifferential misclassification of the exposure. However, daily standardized measurements from the air quality national network (XVPCA, http://dtes.gencat.cat/icqa) were used to temporally adjust the concentration of the air pollutants to each specific period.

The selection of EC and NO_2 as indicators of traffic pollution even though potential synergistic effects of other air pollutants cannot be discarded. Nevertheless, we did not aim to identify the specific neurotoxic agents that are directly responsible for neuronal damage, or to disentangle which of the different agents in the mixture of traffic-related air pollutants causes the observed associations on attentiveness or signs of dyslexia, but we rather used a measure that is globally representative of exposure to traffic pollution. Furthermore, Barcelona is a diesel-dominated vehicle fleet (with very high levels of EC and NO_2 emissions).

Repeated test application over a 1-year period may include some practice effects. However, in previous studies, despite short test-retest intervals (i.e., hours or days) in attentional function in children or in adults the magnitude of the practice effects was moderate to small and the ANT networks showed robustness against practice (Ishigami & Klein, 2011; Mollica, Maruff, Collie, & Vance, 2005). Furthermore, the exact age in each assessment and the time intervals between the neuropsychological testing varied among the children and that contributed to minimizing practice effects.

Attention processes fluctuate daily in response to several individual or environmental factors (Ballard, 1996; Castellanos et al., 2005). However, we included in our analysis factors previously reported as being related to attention fluctuations such as temperature, season, day of the week, or hour of the exam to minimize potential confounding.

Teacher-rated ADHD symptoms are not be confounded with a medical diagnosis of ADHD. Furthermore, attention assessment would have benefited from a multi-informant approach (e.g., teachers and parents).

Warning signs of dyslexia were assessed by the classroom teachers through a 3-4 items questionnaire and are not to be confounded with a clinical diagnosis of dyslexia by a qualified professional. Nevertheless, the items were taken from the PRODISCAT: *Protocol de detecció i actuació en la dislèxia. Àmbit educatiu* (Protocol for detection and management of dyslexia. Educational scope), the current official Catalonian protocol (Col·legi de Logopedes de Catalunya, 2011).

6.4 Future research

Traffic-air pollution seems to cause a detrimental effect on the attention function but the association with ADHD is still not conclusive. More research is needed; traffic noise should be accounted for in the analyses.

Further research should replicate the results encountered in the study of socio-environmental exposures and ADHD symptoms at older ages of the INMA cohort.

Despite the well-described clinical differences between ADHD subtypes, a hypothesized differential influence of the environment is still not confirmed. Studies with larger population size and well defined/assessed outcomes are warranted. Furthermore, the role of gender should be considered.

Regarding covariates, it could be interesting to gather more information about the families such as parental childrearing practices (for its impact on children's health, development, and behavior) (e.g., parental nurturing behaviors, parent-child interaction, or quality of the home environment), family habits (e.g., eating, sleeping, exercise, reading, or screen-time), and family history (e.g., attention difficulties, mental health problems, or medical conditions).

There is strong convergence between human and animals studies regarding the effects of air pollution on the CNS. New policies on air quality are urgently required.

There should be marketing of s*hock* advertising campaigns about the risks of air pollution on humans' health to raise public awareness.

Information and awareness raising campaigns about the risks associated with smoking (also second hand smoke) and particularly gestational drinking are urgently required to protect new generations. Social acceptance of drinking makes it difficult but efforts should be made because they represent many cases of preventable disability.

7. CONCLUSIONS

The main conclusions of this thesis are:

1. We found sufficient evidence for pre- or postnatal exposure to outdoor air pollution and a negative impact on the neuropsychological development of children.

2. We detected an ongoing development of some aspects of attention in primary school children, differentiating patterns by gender and ADHD symptoms. Furthermore, our findings supported the ANT for assessing attention processes in children in large epidemiological studies.

3. Higher daily levels of traffic-related ambient air pollution were associated with higher inattentiveness in primary school children.

4. Prenatal, and at some extent postnatal, exposure to traffic-related air pollution increased the risk of presenting signs of dyslexia in primary school children.

5. In a population-based study, among a large set of socio-environmental factors, only male gender, younger relative age, psychosocial adversity, smoking and prenatal exposure to alcohol were associated with ADHD symptoms at preschool age, pointing towards these would be the most relevant determinants.

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ANNEX

Apart from the original papers included in the present thesis, the PhD candidate has also published other papers as a co-author:

Álvarez-Pedrerol, M., Rivas, I., López-Vicente, M., **Suades-González, E**., Donaire-Gonzalez, D., Cirach, M., de Castro, M., Esnaola, M., Basagaña, X., Dadvand, P., Nieuwenhuijsen, M., Sunyer, J. (2017). Impact of commuting exposure to traffic-related air pollution on cognitive development in children walking to school. *Environmental pollution, 231*, 837-844

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