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## Effects of prenatal exposure to particulate matter air pollution on corpus callosum and behavioral problems in children

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## **ABSTRACT**

### **Objective**

Air pollution (AP) may affect neurodevelopment, but studies about the effects of AP on the growing human brain are still scarce.

We aimed to investigate the effects of prenatal exposure to AP on lateral ventricles (LV) and corpus callosum (CC) volumes in children and to determine whether the induced brain changes are associated with behavioral problems.

### **Methods**

Among the children recruited through a set of representative schools of the city of Barcelona, (Spain) in the Brain Development and Air Pollution Ultrafine Particles in School Children (BREATHE) study, 186 typically developing participants aged 8-12 years underwent brain MRI on the same 1.5 Tesla MR unit over a 1.5-year period (October 2012 - April 2014). Brain volumes were derived from structural MRI scans using automated tissue segmentation. Behavioral problems were assessed using the Strengths and Difficulties Questionnaire (SDQ) and the criteria of the Attention Deficit Hyperactivity Disorder DSM-IV list. Prenatal fine particle (PM<sub>2.5</sub>) levels were retrospectively estimated at the mothers' residential addresses during pregnancy with land use regression (LUR) models. To determine whether brain structures might be affected by prenatal PM<sub>2.5</sub> exposure, linear regression models were run and adjusted for age, sex, intracranial volume (ICV), maternal education, home socioeconomic vulnerability index, birthweight and mothers' smoking status during pregnancy. To test for associations between brain changes and behavioral outcomes, negative binomial regressions were performed and adjusted for age, sex, ICV.

### **Results**

Prenatal PM<sub>2.5</sub> levels ranged from 11.8 to 39.5 μg/m<sup>3</sup> during the third trimester of pregnancy. An interquartile range increase in PM<sub>2.5</sub> level (7 μg/m<sup>3</sup>) was significantly linked to a decrease in the body CC volume (mm<sup>3</sup>) (β=-53.7, 95%CI [-92.0, -15.5] corresponding to a 5% decrease of the mean body CC volume) independently of ICV, age, sex, maternal education, socioeconomic vulnerability index at home, birthweight and mothers' smoking status during the third trimester of pregnancy. A 50 mm<sup>3</sup> decrease in the body CC was associated with a significant higher hyperactivity subscore (Rate Ratio (RR) = 1.09, 95%CI [1.01, 1.17] independently of age, sex and ICV. The statistical significance of these results did not survive to False Discovery Rate correction for multiple comparisons.

### **Conclusions**

Prenatal exposure to  $PM_{2.5}$  may be associated with CC volume decrease in children. The consequences might be an increase in behavioral problems.

**KEYWORDS:** Neuroimaging, corpus callosum, lateral ventricle, behavioral problems, air pollution, children.

# 1. Introduction

Air pollution is a complex mixture of particulate matter (PM), gases, organic volatile compounds and metals produced by combustion of fossil fuels and industrial and agricultural processes.

Over the past decades, air pollution has emerged as a potential disruptor of neurodevelopment in children and has been associated with a higher prevalence of Attention Deficit Hyperactivity Disorder (ADHD) and autism spectrum disorder (ASD) (Suades-González et al., 2015). However, studies about the potential damages on the growing brain induced by chronic exposure to air pollution are still scarce. We recently showed in a large sample of schoolchildren (Sunyer et al., 2015) that higher traffic-related air pollution (TRAP) exposure during childhood was associated with lower functional integration in key brain network but not linked with any structural alteration (Pujol et al., 2016). Conversely, two neuroimaging studies assessing levels of prenatal exposure to air pollution observed that, in pre-adolescent children, such an exposure was associated with thinner cortex in several brain regions of both hemispheres (Guxens et al., 2018) and with substantial reductions in white matter (WM) surface, independently of levels of air pollution encountered during childhood (Peterson et al., 2015).

Prenatal exposure to high levels of air pollution may have more severe consequences for the developing brain than the exposure during childhood. Firstly because brain structures are forming during this period and then, because the effect of in utero exposure to environmental factors may cause permanent brain injury and predict cognitive impairment later in life (Lee et al., 2017). Supporting the importance of the prenatal period, a series of mice experiments consistently reported that pre- and neonatal exposure to fine PM and diesel exhaust particles induced inflammatory reactions in the corpus callosum (CC) (Allen et al., 2015; Klocke et al., 2017; Morris-Schaffer et al., 2019), the major WM tract in the brain, suggesting that the developing WM might be a target of PM toxicity (Klocke et al., 2018). Moreover, these inflammatory responses to PM exposure were accompanied by modifications in the CC size, aberrant myelination and enlargement of the cerebral lateral ventricles (LV) (Allen et al., 2015, 2014; Klocke et al., 2017). These observations in mice are of particular translational significance given that these brain structures alterations are morphological features commonly reported in neurodevelopmental disorders with behavioural manifestations such as ADHD and ASD (Cao et al., 2010; Dougherty et al., 2016; Lefebvre et al., 2015; Lyoo et al., 1996; Wolff et al., 2015). In addition, early-life exposure to PM has been found associated with an increased risk of ADHD (Aghaei et al., 2019) and ASD (Flores-Pajot et al., 2016).

However, whether the effects of prenatal exposure to air pollution on CC and LV observed in mice are similar in humans remains to determine. The main aim of this study was to investigate

38 the effects of prenatal exposure to fine PM on WM, gray matter (GM), LV and CC in typically  
39 developing children from the general population, using a neuroimaging regions of interest (ROI)  
40 approach with the a priori hypothesis that higher prenatal exposure to fine PM would be associated  
41 with reduced CC volume, and higher LV volumes. Secondly, we investigated whether reduced  
42 CC volume and higher LV volume were associated with behavioral problems in our population.

43

44

## 45 **2. Methods**

### 46 **2.1 Study design and participants**

47 This study was developed in the context of the Brain Development and Air Pollution Ultrafine  
48 Particles in School Children (BREATHE) project (Sunyer et al., 2015). Forty schools in  
49 Barcelona (Catalonia, Spain) were selected based on modeled traffic-related nitrogen dioxide  
50 (NO<sub>2</sub>) values. Low- and high -NO<sub>2</sub> schools were paired by socioeconomic vulnerability index and  
51 type of school (i.e., public/private). Between 2012 and 2013, a total of 39 representative schools  
52 of the city of Barcelona and 2897 children agreed to participate in the whole survey.

53 All participating children were given a document asking whether they were interested in the MRI  
54 study. The document was returned by 1564 families and among them, 810 gave an initial positive  
55 response. Parents of 491 children were successfully contacted. Consent to participate was not  
56 obtained in 165 cases, 27 children were lost before the MRI assessment, 21 were excluded because  
57 of dental braces, 34 had brain scans of poor quality and 58 had missing data (Figure 1). The final  
58 MRI sample included 186 children (49% of girls, median age at MRI of 9.7 years).

59 All parents or tutors signed the informed consent form approved by the Research Ethical  
60 Committee (No. 2010/41221/I) of the IMIM-Parc de Salut Mar., Barcelona, Spain and the FP7-  
61 ERC-2010-AdG Ethics Review Committee (268479-22022011).

62

### 63 **2.2 Exposure to fine PM**

64 From the prenatal period to the time of the MRI study, the history of exposures to PM with an  
65 aerodynamic diameter <2.5µm (PM<sub>2.5</sub>) were estimated at the geocoded postal address of each  
66 participant using land use regression (LUR) models (Eeftens et al., 2012). The area-specific LUR  
67 models of annual PM<sub>2.5</sub> used in this study were developed within the framework of the European  
68 Study of Cohorts for Air Pollution Effects (ESCAPE, [www.escapeproject.eu](http://www.escapeproject.eu)). Briefly, PM were  
69 measured between October 2008 and April 2011 at twenty sampling sites in Barcelona. These  
70 measurements were then temporally corrected to estimate annual average concentrations in the  
71 Barcelona area (Eeftens et al., 2012). The Geographic information systems-derived variables  
72 which better predicted spatial variation of these annual average concentrations of PM in the  
73 Barcelona area were i) land-use (sum of Urban green and semi-natural and forested areas in a  
74 100m<sup>2</sup> buffer size area), traffic-related (traffic intensity on nearest road in vehicules.day<sup>-1</sup> x

75 inverse squared distance to the nearest road, and total traffic load of all roads in a buffer of 100m)  
76 for the PM<sub>2.5</sub> LUR models, ii) traffic-related (traffic intensity on nearest road in vehicules.day<sup>-1</sup> x  
77 inverse distance to the nearest road, and road length of all roads in a buffer of 25m), and altitude  
78 variables for the PM<sub>10</sub> LUR models.

79 Because the measurement periods in ESCAPE did not overlap with the time windows of exposure  
80 we wished to explore, residence-based exposures derived from the LUR models were then  
81 adjusted to the specific periods of time of interest in this study using daily PM records from an  
82 urban background station from the air quality national network (XVPCA,  
83 <http://dtes.gencat.cat/icqa>) covering the study area. PM<sub>2.5</sub> was started to be monitored in  
84 Barcelona in 2008-2009, until then there are no records. The temporal modelling of PM<sub>2.5</sub> for any  
85 time window prior to 2008 was conducted consequently with the PM<sub>10</sub> routine monitoring data.  
86 A known ratio between PM<sub>10</sub>/PM<sub>2.5</sub> was applied.

87

88 Parents reported the history of residence of the child via questionnaire where they indicated the  
89 periods that the family lived in each residence in case they moved. If they moved, we calculated  
90 the time-weighted average of the PM<sub>2.5</sub> concentration estimated for each residence within the year.  
91 In cases of separated parents with shared custody, the participants were assigned the time  
92 averaged exposure according to the time that they spent in each home. We did not have the history  
93 of residences during the pregnancy period. We assumed the first residence of the child was the  
94 same during the pregnancy period.

95 Following this procedure, PM<sub>2.5</sub> exposures were estimated i) for the whole pregnancy, ii) for the  
96 first, the second and the third trimesters of pregnancy, iii) for the 2 first years of life, iv) at the  
97 time of the MRI study. The pregnancy period, as well as the 3 trimesters of pregnancy were  
98 determined using the children's birth dates and gestational ages in weeks. Children for whom the  
99 gestational age was unknown (9 missing data, 3 adopted children, 19 preterm children with  
100 gestational age <37 weeks) were excluded from the analysis sample.

101

102 Prenatal PM<sub>2.5</sub> levels were the exposure variables used in the main analyses. We also considered  
103 PM<sub>2.5</sub> exposure during the 2 first years of life and PM<sub>2.5</sub> exposure at the time of the MRI study in  
104 sensitive analyses.

105

## 106 **2.3 MRI**

107

### 108 2.3.1 MRI acquisition

109 Cerebral anatomical images of the entire surface of the brain were acquired between October 2012  
110 and April 2014. In order to minimize movement in the scanner, children were asked to stand still  
111 once in the machine. A 1.5-Tesla Signa Excite system (General Electric, Milwaukee, WI)

112 equipped with an eight-channel phased-array head coil was used. Anatomical images were  
113 obtained using an axial T1-weighted three-dimensional (3D) fast spoiled gradient inversion  
114 recovery prepared sequence. A total of 134 contiguous slices were acquired with repetition time  
115 11.9 msec, echo time 4.2 msec, flip angle 15°, field of view 30 cm, 256 x 256 pixel matrix, and  
116 slice thickness 1.2 mm. Total acquisition time was 5mn 40s for the 3D sequence.

117

### 118 2.3.2 Data preprocessing and segmentation

119 Cortical reconstruction and volumetric segmentation were performed with the version 5.3 of the  
120 Freesurfer image analysis suite (<http://surfer.nmr.mgh.harvard.edu/>). The technical details of  
121 these procedures are described in prior publications (Dale et al., 1999; Fischl et al., 2004, 2002,  
122 1999a; Ségonne et al., 2004).

123 Briefly, this processing includes removal of non-brain tissue using a hybrid watershed/surface  
124 deformation procedure (Ségonne et al., 2004), automated Talairach transformation, segmentation  
125 of the subcortical white matter and deep gray matter volumetric structures (including  
126 hippocampus, amygdala, caudate, putamen, ventricles) (Fischl et al., 2004, 2002) intensity  
127 normalization (Sled et al., 1998), tessellation of the gray matter white matter boundary, automated  
128 topology correction (Fischl et al., 2001; Ségonne et al., 2007), and surface deformation following  
129 intensity gradients to optimally place the gray/white and gray/cerebrospinal fluid borders at the  
130 location where the greatest shift in intensity defines the transition to the other tissue class (Dale  
131 et al., 1999).

132 Once the cortical models were complete, registration to a spherical atlas which is based on  
133 individual cortical folding patterns to match cortical geometry across subjects (Fischl et al.,  
134 1999b) was performed.

135

### 136 2.3.3 Quality control

137 All the anatomical images were visually inspected by a trained researcher before and after the  
138 pre-processing steps. Right after acquisition, cases were systematically excluded based on expert  
139 subjective criteria if the raw images showed obvious motion artefacts (ghost and blurring of the  
140 image), ringing or truncation artefacts, and susceptibility phenomena. No scan repetition was  
141 done. After preprocessing, cases were excluded if the images showed deformation of the three-  
142 dimensional brain anatomy and large truncated brain areas, non optimal removal of nonbrain  
143 tissue, and obvious tissue (Gray and White matter) misclassification.

144

### 145 2.3.4 Neuroimaging variables

146 Volumetric regions from both hemispheres were averaged. The segmentation of the CC provided  
147 the volume of several anatomic regions: CC posterior, CC mid-posterior, CC central, CC mid-  
148 anterior, CC anterior. CC mid-posterior, CC central and CC mid-anterior volumes were summed

149 up to give the volume of the body CC. The volume of the total CC was obtained by summing up  
150 all the anatomical regions.

151 Intracranial volume (ICV) was also estimated and was further used as an adjustment factor in the  
152 statistical analyses to take brain size into account.

153

## 154 **2.5 Behavioral outcomes**

155 The Strengths and Difficulties Questionnaire (SDQ) is widely used as an international  
156 standardized instrument measuring child behavior (Goodman, 1997). The SDQ, filled out by  
157 parents in our study, comprises five separate subscales, each including 5 questions covering  
158 different behavioral aspects including emotional symptoms, conduct problems,  
159 hyperactivity/inattention, peer relationship problems, and prosocial behavior. Each question is  
160 rated on a 3-point scale [not true (0), somewhat true (1), and certainly true (2)]. A total difficulties  
161 score (ranging from 0 to 40) is generated by summing the scores for all subscales except the  
162 prosocial behavior scale (considered a behavioral strength). We analysed the total difficulties  
163 score as a continuous variable.

164 ADHD symptomatology was reported by teachers using the ADHD/ Diagnostic and Statistical  
165 Manual of Mental Disorders (DSM-IV) Scale, which consists of a list of 18 symptoms categorized  
166 under two separate symptom groups: inattention (nine symptoms) and hyperactivity/impulsivity  
167 (nine symptoms). Each ADHD symptom is rated on a 4-point scale (0 = never or rarely, 1 =  
168 sometimes, 2 = often, or 3 = very often).

169 ADHD scale and SDQ questionnaires were filled out by teachers and parents between September  
170 2012 and June 2013. Higher SDQ total difficulties score and/or higher ADHD scores indicate  
171 more behavioral problems.

172

## 173 **2.6 Contextual and Individual Covariates**

174 Socio-demographic factors were measured using a neighborhood socio-economic vulnerability  
175 index (based on level of education, unemployment, and occupation in each census tract, the finest  
176 spatial census unit, with median area of 0.08 km<sup>2</sup>) (Ministry of Public Works, 2012) according to  
177 both school and home addresses, and maternal education ( $\leq$  than primary, secondary or university)  
178 (Sunyer et al., 2015).

179 We also considered birthweight (<2.5kg, 2.5-4kg or >4kg) and mothers' smoking status during  
180 pregnancy (Smoking, No smoking).

181

## 182 **2.7 Statistical analyses**

183 To determine whether brain structures might be affected by prenatal PM<sub>2.5</sub> exposure, we tested  
184 separate linear regression models for each neuroimaging variables as dependent variables (GM,  
185 WM, CC, and LV volumes) and prenatal (three trimesters and entire pregnancy) PM<sub>2.5</sub> levels as

186 independent variables. The analyses were adjusted for age, sex, ICV, maternal education, home  
187 socioeconomic vulnerability index, birthweight and mothers' smoking status during pregnancy.  
188 Interaction effects between PM<sub>2.5</sub> exposure levels and sex, and between PM<sub>2.5</sub> exposure levels and  
189 age at the time of MRI, were examined including interaction terms in the regression models.

190

191 To test for associations between behavioral outcomes and brain volumes, we fit negative binomial  
192 regression in order to account for the overdispersion in the ADHD and SDQ scores (eFigure 1).  
193 Analyses were adjusted for age, sex, ICV.

194

195 To account for the fact that we examined multiple brain regions and multiple time windows of  
196 exposure, we adjusted significance levels using the false discovery rate (FDR) method (Benjamini  
197 and Hochberg, 1995). Analyses were run using Stata 14 (StataCorp). Statistical significance was  
198 set at  $p < 0.05$ .

199

#### 200 *Sensitivity analyses*

201 Given that the participating children were recruited through schools, we ran linear mixed models  
202 with school as nested random effect to verify that the multilevel nature of the data did not  
203 influence the results.

204 We also ran regression linear models including exposures in all of the trimesters of pregnancy to  
205 determine which trimester may be the most predictive on the brain outcomes.

206 Then, we examined the relationships between prenatal PM<sub>2.5</sub> exposure and brain structures with  
207 an additional adjustment for current PM<sub>2.5</sub> exposure.

208 Finally, we investigated the relationship between PM<sub>2.5</sub> exposure during the 2 first years of life  
209 and the brain volumes.

210

211

### 212 **3. Results**

213

214 Forty-nine percent of children were girls and age at MRI ranged between 8.0 and 11.7 years  
215 (Table 1). Characteristics of the MRI subsample were similar to those of the overall BREATHE  
216 sample, but for home socioeconomic index with less deprivation in the MRI subsample (eTable  
217 1).

218 Prenatal PM<sub>2.5</sub> levels across the three trimesters of pregnancy had similar and normal distributions  
219 (eFigure 2) and were slightly variable (medians ranged from 22.8 to 24.1 $\mu\text{g}/\text{m}^3$ ). Pearson's  
220 correlation coefficients between PM<sub>2.5</sub> levels across the three trimesters of pregnancy ranged from  
221 0.75 to 0.77 (eTable 2).

222

223 **3.1 Prenatal exposure to PM<sub>2.5</sub> and brain volumes**

224 Table 2 presents the adjusted PM<sub>2.5</sub> coefficients for the neuroimaging outcomes. Independently  
225 of ICV, age, sex, maternal education, socioeconomic vulnerability index at home, birthweight and  
226 mother's smoking status during pregnancy, an interquartile range increase in PM<sub>2.5</sub> levels during  
227 the third trimester of pregnancy (7 µg/m<sup>3</sup>) was significantly linked to a decrease in the anterior  
228 and the body CC volumes ( $\beta = -24.8$ , 95%CI [-48.7, -0.95],  $\beta = -53.7$ , 95%CI [-92.0, -15.5]  
229 respectively). However, the statistical significance of these associations did not survived FDR  
230 correction for multiple comparisons (Benjamini-Hochberg correction, (Benjamini and Hochberg,  
231 1995)).

232 No associations were observed between PM<sub>2.5</sub> and the other neuroimaging variables (LV, GM  
233 and WM).

234 No significant interaction between sex and prenatal exposure to PM<sub>2.5</sub>, nor between age and  
235 prenatal exposure to PM<sub>2.5</sub>, were observed (eTable 5).

236

237 **3.2 Brain volumes and behavioral problems**

238 Significant higher hyperactivity subscores were observed for a 50 mm<sup>3</sup> decrease in the body CC  
239 (Rate Ratio (RR) = 1.09, 95%CI [1.01, 1.17], corresponding to a subscore 9% increase,  
240 independently of age, sex and ICV (Table 3). However, the statistical significance of these  
241 associations did not survive FDR correction.

242

243 **3.3 Sensitivity analyses**

244 Taking into account the multilevel nature of the data in linear mixed models with school as nested  
245 random effect did not influence the results (data not shown).

246 In the linear regression models including simultaneously exposures in all of the trimesters of  
247 pregnancy, higher exposure during the 3rd trimester remained significantly associated to decrease  
248 in CC volumes (eTable 4).

249 Additional adjustment for current PM<sub>2.5</sub> exposure at home did not change the relationships  
250 between prenatal exposure to PM<sub>2.5</sub> and CC volumes (eTable 6).

251 The investigation of the relationship between exposure to PM<sub>2.5</sub> during the 2 first years of life and  
252 brain volumes did not reveal any significant impact of PM<sub>2.5</sub> exposure on the CC and LV volumes  
253 during this period (eTable 7).

254

255

256 **4. Discussion**

257

258 We examined the relationship between prenatal exposure to PM<sub>2.5</sub> and CC and LV volumes later  
259 in childhood. In our sample, we observed that prenatal exposure to PM<sub>2.5</sub>, particularly during the  
260 last trimester of pregnancy, may induce structural changes in the CC in children aged between 8-

261 12 years from the general population. An increase of approximately  $7\mu\text{g}/\text{m}^3$  in  $\text{PM}_{2.5}$  level during  
262 the third trimester of pregnancy was indeed associated with a reduction corresponding to almost  
263 5% of the mean volume of the body CC. Brain changes of this magnitude may result in an increase  
264 in ADHD symptoms (hyperactivity), as well as in more general behavior problems.

265  
266 CC is composed of mostly myelinated axons, forming the largest WM bundle in the brain. It  
267 connects homologous areas of the two cerebral hemispheres and is essential for communication  
268 between them (Banich, MT, 2003). Structural alterations of the CC are frequently observed in  
269 ADHD and ASD (Cao et al., 2010; Lefebvre et al., 2015; Lyoo et al., 1996; Wolff et al., 2015).  
270 The prenatal  $\text{PM}_{2.5}$  exposure-related reduction in CC volume observed in our study is possibly the  
271 consequence of changes in the number or size of axons connecting the two hemispheres, but may  
272 also reflect differences in the number of cortical neurons within homologous regions. Both  
273 neurons and oligodendrocytes (cells in charge of producing the myelin sheath in the central  
274 nervous system) proved to be vulnerable to TRAP exposure in experimental studies (Block and  
275 Calderón-Garcidueñas, 2009; Ejaz et al., 2014; Woodward et al., 2017). This toxicity to TRAP is  
276 thought to be mediated by microglia, the resident immune cells in the brain (Genc et al., 2012).  
277 Indeed, TRAP exposure appears to trigger an overactivation of microglia which then release  
278 factors (reactive oxygen species, cytokines and tumor necrosis factor) toxic for neurons and  
279 oligodendrocytes (Genc et al., 2012). Microglia's receptors are reachable by different ways. After  
280 inhalation and from the lungs,  $\text{PM}_{2.5}$  can directly translocate to the systematic circulation and/or  
281 provoke the release of circulating inflammatory agents. Both  $\text{PM}_{2.5}$  and inflammatory agents are  
282 then likely to cross the blood brain barrier. Regarding the prenatal period, TRAP may impair the  
283 placenta function (van den Hooven et al., 2012) which in turn could disrupt foetal  
284 neurodevelopment (Batalle et al., 2016; Egaña-Ugrinovic et al., 2015; Eixarch et al., 2016). In  
285 addition, experimental evidence for transplacental transfer of nano-size PM (Takeda et al., 2009)  
286 and maternal cytokines (Dahlgren et al., 2006) suggest that PM inhaled by the mother could also  
287 overactivate microglia in the foetus' brain.

288 The study's volumetric ROI approach does not permit to determine the nature of the  
289 microstructural changes leading to the reduction of the CC volume. In order to investigate this  
290 question, we ran in parallel whole brain Voxel-Based Morphometry (VBM) and Diffusion Tensor  
291 Imaging (DTI) analyses in a naïve approach within the MRI subsample. However, at the voxel  
292 level, we did not observe any structural changes linked to prenatal exposure to  $\text{PM}_{2.5}$  during the  
293 1st, the 2nd nor the 3rd trimester of pregnancy (Supplemental Material).

294 With the volumetric ROI approach, we nevertheless observed that the effects on the children's  
295 CC volume were the most severe during the third trimester of pregnancy. This observation is in  
296 coherence with results from animal studies which report reduction in CC size after exposure to  
297 particle during a developmental window of CNS development in mice equivalent to human 3<sup>rd</sup>

298 trimester (Allen et al., 2015) (Clancy et al., 2007) but not for earlier exposures equivalent to  
299 human 1<sup>st</sup> and 2<sup>nd</sup> trimesters (Klocke et al., 2017). Moreover, besides a reduction of the CC area,  
300 mice presented hypomyelination and aberrant WM development (Allen et al., 2015). These  
301 observations, along with the fact that late gestation in humans is the seat of oligodendrocytes  
302 maturation (Dubois et al., 2014), suggest that the effects on CC reported in the context of our  
303 study are more likely to be the consequences of the PM<sub>2.5</sub> toxicity towards oligodendrocytes rather  
304 than neurons. This hypothesis is supported by the absence of changes in GM volume, similarly to  
305 the findings from the Peterson's study showing that high levels of TRAP during the third trimester  
306 of pregnancy were associated with substantial reduction in WM surface but not with cortical  
307 thickness in 40 children 7-9 years of age (Peterson et al., 2015). Following the studies of Allen  
308 (Allen et al., 2015) and Peterson (Peterson et al., 2015) that point at late gestation as a particularly  
309 sensitive windows for the association between TRAP and WM development, we did not observe  
310 any significant impact on the CC for exposure to PM<sub>2.5</sub> during the 2 first years of life and  
311 adjustment for current exposure to PM<sub>2.5</sub> did not change the magnitude of the associations.  
312 In addition, in the same participants and using VBM analyses, we recently reported no  
313 associations between TRAP exposure during childhood and brain anatomy (Pujol et al., 2016). In  
314 parallel to structural brain changes, a recent retrospective study on several cognitive functions in  
315 children suggests that the period from week 20 onwards of gestation could be the most vulnerable  
316 to TRAP exposure (Chiu et al., 2016). As well, a recent meta-analysis of studies investigating the  
317 associations between prenatal PM<sub>2.5</sub> exposure and risk of ASD showed that the strength of the  
318 association increased in parallel with the pregnancy advancement (Flores-Pajot et al., 2016).  
319  
320 The magnitude of the effects, a 5% decrease of the mean body CC volume for an interquartile  
321 range increase in prenatal PM<sub>2.5</sub> levels, might be of concern for several reasons. Firstly, because  
322 these effects were observed for a chronic prenatal exposure to PM<sub>2.5</sub> at levels that did not exceed  
323 the limit target value of 25 µg/m<sup>3</sup> established by the European Union for 2015 (Directive  
324 2008/50/EC). It is important to highlight that in the Allen's study (Allen et al., 2015), the  
325 deleterious effect of ultrafine particles on mice's CC were also reported for levels consistent with  
326 high traffic areas of major U.S. cities and consequently likely to be encountered by the general  
327 population. Secondly because, even though not specific to these neurodevelopmental disorders,  
328 CC reductions are commonly reported in ADHD and ASD (Dougherty et al., 2016). For example,  
329 the mean CC volume was reported to be 12% smaller in children with ASD compared with healthy  
330 controls (Frazier et al., 2012). Finally, higher hyperactivity score was observed for CC volume  
331 reduction estimated for a 7µg/m<sup>3</sup> increase in PM<sub>2.5</sub> levels during the late prenatal life. These last  
332 findings provide additional information to the Allen's study which did not investigate the  
333 behavioral consequences of the reported air pollution-related reduction in CC area. However, in  
334 contrast to mice experiments, we did not observe significant increase of LV volume in children.

335

336 In this study, PM<sub>2.5</sub> was targeted because, of all air pollutants, particles are thought to be the most  
337 important inhaled toxicants in urban air (Kaiser, 2005), particularly in relation to brain damage  
338 (González-Maciel et al., 2017). Besides, mice experiments reported effects of ultrafine PM on  
339 CC, possibly through iron toxicity (Klocke et al., 2017).

340

341 Several limitations in this study must be considered. Firstly, comparison of the characteristics of  
342 the MRI subsample population and the overall BREATHE population showed that home  
343 socioeconomic vulnerability index was significantly different between groups, with less  
344 deprivation in the MRI subsample. However, this probably does not affect the possibility of  
345 extrapolating the results to the overall BREATHE study since i) the distribution of the exposure  
346 and behavioral variables are not significantly different between the overall BREATHE cohort and  
347 the MRI subsample, ii) the index was not significantly associated with the neuroimaging variables  
348 and the behaviour assessments.

349 Secondly, we cannot rule out that the findings presented here are false positive since their  
350 statistical significance did not survive to FDR correction for multiple testing. However, it might  
351 be just as troublesome, from a public health perspective, to conclude that there is no relationship  
352 between prenatal exposure to PM<sub>2.5</sub> and CC volume because the Benjamini-Hochberg false  
353 discovery rate of 5% might be too high and not adequate in this context, since there is correlation  
354 among all the CC structures, as well as among the trimesters specific exposures to PM<sub>2.5</sub>, and for  
355 all of them the association was negative. In addition, whole brain VBM and DTI analyses did not  
356 support the volumetric findings. However, it is important to highlight that such analyses are also  
357 subject to correction for multiple comparisons rather restrictive given the number of voxels and  
358 consequently of tests performed.

359 Probably because of insufficient power, we did not observe any statistically significant  
360 associations between prenatal PM<sub>2.5</sub> exposure and behavioral outcomes while we found such an  
361 association in previous work in the whole population of the BREATHE study (Forns et al., 2016).  
362 We were consequently not able to investigate whether the CC volume decrease was mediating  
363 any link between PM<sub>2.5</sub> and behavioral problems.

364 Then, we only used ambient modelled PM<sub>2.5</sub> estimates and did not have personal monitoring nor  
365 biomarkers, thus our PM<sub>2.5</sub> levels only provide indirect estimates of the real exposure to PM<sub>2.5</sub>.  
366 Furthermore, we assumed that the first residence of the child reported in the residential history  
367 questionnaire was the same during the pregnancy period. These aspects of the exposure  
368 assessment method used in this study may have led to non-differential exposure misclassification  
369 and in turn to regression coefficients closer to the null value that they probably are.

370 Finally, TRAP is actually a mixture of environmental contaminants and the effects observed here  
371 might be not only attributable to PM<sub>2.5</sub>.

372 Even though the effects of PM<sub>2.5</sub> we observed were more severe on brain structures for higher  
373 exposure during the third trimester of pregnancy, our study design do not permit to disentangle  
374 the effects of potential susceptibility windows. As well, we cannot neither rule out that this result  
375 was driven by a cumulative exposure effect throughout the 3 trimesters of pregnancy. Our findings  
376 only add up to an amount of observations suggesting that pregnancy is a particularly crucial period  
377 (Allen et al., 2015; Flores-Pajot et al., 2016).

378 Eventually, information about children's Intelligence Quotient and other deleterious prenatal  
379 exposures such as noise, that are both associated to PM<sub>2.5</sub> levels and to neurobehavioral problems  
380 in children (Chiu et al., 2016; Forns et al., 2016; Sandtorv et al., 2018), were not available. We  
381 were consequently not able to consider these potential confounders in the analyses. It could have  
382 affected the accuracy of the regression parameters and rate ratios that we observed.

383

## 384 **5. Conclusions**

385 In conclusion, this study suggests that prenatal exposure to PM<sub>2.5</sub> is associated with changes on  
386 the CC volume in pre-adolescent children, even for levels not exceeding the limit target values  
387 established in the European Union. We have replicated the experimental findings in mice using a  
388 neuroimaging Region of Interest approach, but we went beyond by showing that the consequences  
389 of this air pollution-related CC volume reduction might be an increase in behavioral problems.  
390 Future larger and prospective studies are needed to confirm these findings in children.

### **Conflict of interest**

The authors declare that they have no conflict of interest.

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## **Ethical approval**

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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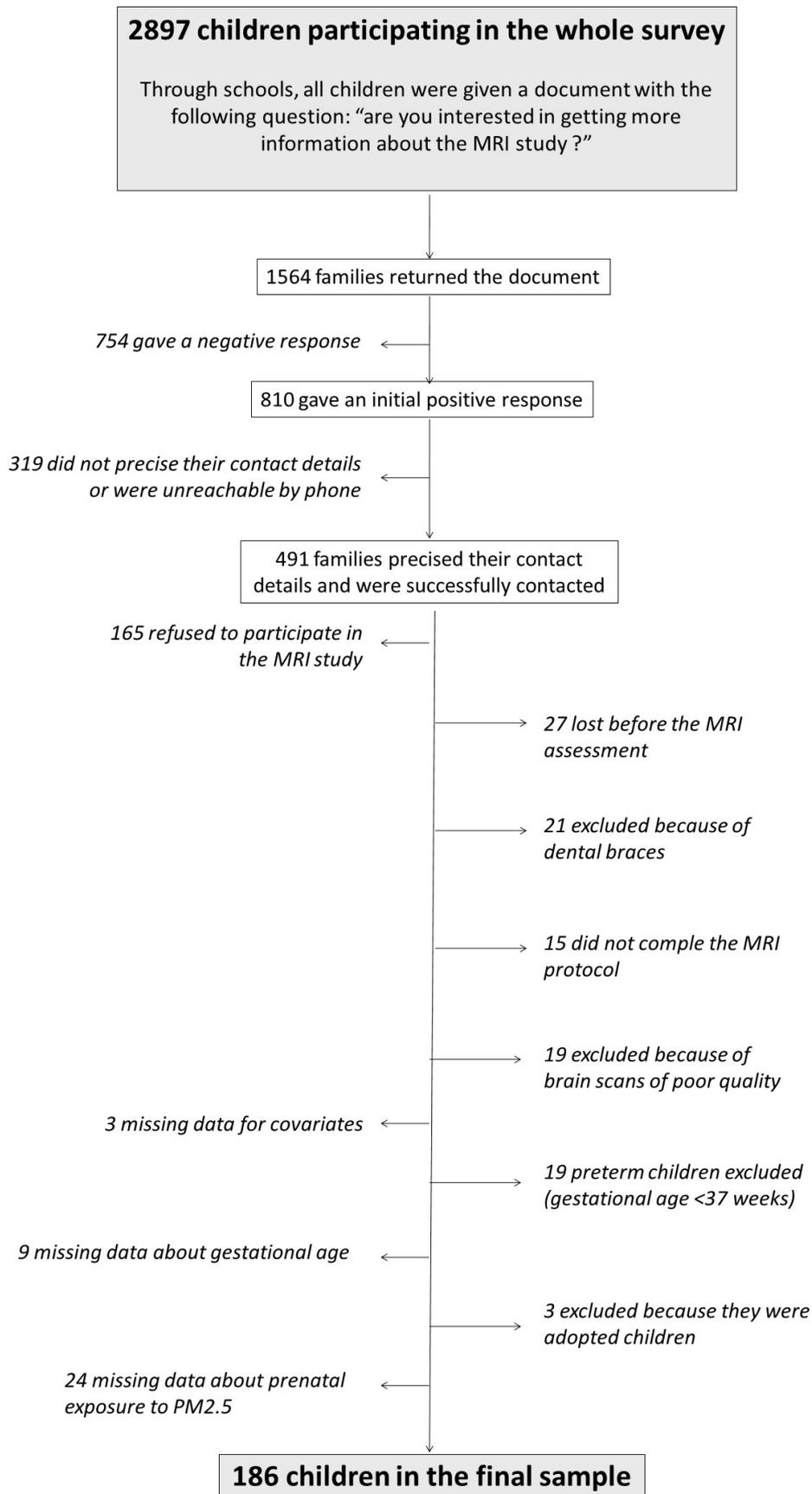
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**Figure 1. Flow chart**

**Table 1. Children's characteristics, n=186**

| <b>Socio-demographic characteristics</b>                        |                         |
|---|-------------------------|
| Age at MRI, yrs (median, range)                                 | 9.7 (8.0, 11.7)         |
| Sex (n, %)  |                         |
| Girls   | 91 (49%)                |
| Boys  | 95 (51%)                |
| Home socioeconomic vulnerability index (median, range)          | 0.39 (0.06, 0.90)       |
| Maternal Education level (n, %)                                 |                         |
| Primary or less than primary                                    | 16 (9%)                 |
| Secondary   | 50 (27%)                |
| University  | 120 (64%)               |
| School attended (n, %)  |                         |
| Private   | 109 (59%)               |
| Public  | 77 (41%)                |
| Birthweight (n, %)  |                         |
| < 2.5kg   | 7 (4%)                  |
| 2.5 – 4kg   | 164 (88%)               |
| > 4kg   | 15 (8%)                 |
| Mother's smoking status during pregnancy (n, %)                 |                         |
| Smoking   | 13 (7%)                 |
| No smoking  | 173 (93%)               |
| <b>Brain structures (median, range)</b>                         |                         |
| ICV, cm <sup>3</sup>  | 1397 (1109, 1752)       |
| White matter, mm <sup>3</sup>                                   | 404188 (314938, 549909) |
| Grey matter, mm <sup>3</sup>                                    | 693236 (541002, 845761) |
| Corpus callosum, mm <sup>3</sup>                                |                         |
| Total   | 2459 (1209, 3729)       |
| Anterior  | 703 (288, 1211)         |
| Body  | 1016 (606, 1490)        |
| Posterior   | 741 (316, 1112)         |
| Lateral ventricles, mm <sup>3</sup>                             | 7500 (2592, 32383)      |
| <b>Behavior (median, range)</b>                                 |                         |
| ADHD DSM-IV scale <sup>a</sup>                                  |                         |
| ADHD total score (n=181)  | 4 (0, 42)               |
| Inattention score (n=182)                                       | 2 (0, 24)               |
| Hyperactivity score (n=182)                                     | 1 (0, 23)               |
| SDQ   |                         |
| Total difficulties score  | 8 (0, 24)               |
| <b>PM<sub>2.5</sub> levels, µg/m<sup>3</sup>(median, range)</b> |                         |
| 1 <sup>st</sup> trimester of pregnancy                          | 24.1 (14.0, 49.0)       |
| 2 <sup>nd</sup> trimester of pregnancy                          | 23.7 (12.2, 47.5)       |
| 3 <sup>rd</sup> trimester of pregnancy                          | 22.8 (11.8, 39.5)       |
| Whole pregnancy   | 23.6 (12.7, 45.0)       |

Abbreviations: ICV, intracranial volume; ADHD, Attention-Deficit Hyperactivity Disorder; SDQ, Strengths and Difficulties Questionnaire; PM<sub>2.5</sub>, Particle Matter with a diameter of 2.5 µm or less.

<sup>a</sup> 5 teachers gave back incomplete ADHD DSM-IV questionnaires

**Table 2. Difference (95% CI) in brain volumes by prenatal air pollution exposure to PM<sub>2.5</sub> (interquartile range increase) in 186 children.**

|                                | PM <sub>2.5</sub> exposure periods |                |                               |                           |                |                               |                           |                |                               |                      |                |                               |
|--------------------------------|------------------------------------|----------------|-------------------------------|---------------------------|----------------|-------------------------------|---------------------------|----------------|-------------------------------|----------------------|----------------|-------------------------------|
|                                | 1 <sup>st</sup> Trimester          |                |                               | 2 <sup>nd</sup> Trimester |                |                               | 3 <sup>rd</sup> Trimester |                |                               | Whole pregnancy      |                |                               |
| <b>Volumes, mm<sup>3</sup></b> | $\beta$ (95%CI)                    | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> | $\beta$ (95%CI)           | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> | $\beta$ (95%CI)           | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> | $\beta$ (95%CI)      | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> |
| Grey Matter                    | 2890 (-1508, 7287)                 | 0.196          | 0.464                         | 1835 (-3272, 6942)        | 0.479          | 0.583                         | 1124 (-4700, 6948)        | 0.704          | 0.800                         | 2094 (-2562, 6750)   | 0.376          | 0.526                         |
| White Matter                   | 215.7 (-3536, 3967)                | 0.910          | 0.910                         | -200.9 (-4543, 4141)      | 0.859          | 0.891                         | -2226 (-7162, 2710)       | 0.375          | 0.526                         | -582.6 (-4544, 3379) | 0.772          | 0.831                         |
| Corpus callosum                |                                    |                |                               |                           |                |                               |                           |                |                               |                      |                |                               |
| Total                          | -48.7 (-106.2, 8.7)                | 0.096          | 0.361                         | -35.6 (-102.4, 31.2)      | 0.295          | 0.504                         | -93.9 (-169.0, -18.8)     | 0.015          | 0.210                         | -57.2 (-117.7, 3.4)  | 0.064          | 0.299                         |
| Anterior                       | -9.5 (-27.7, 8.8)                  | 0.306          | 0.504                         | -9.1 (-30.2, 12.0)        | 0.397          | 0.529                         | -24.8 (-48.7, -0.95)      | 0.042          | 0.294                         | -13.6 (-32.9, 5.6)   | 0.164          | 0.464                         |
| Body                           | -28.9 (-58.3, 0.36)                | 0.053          | 0.297                         | -22.3 (-56.4, 11.8)       | 0.199          | 0.464                         | -53.7 (-92.0, -15.5)      | 0.006          | 0.168                         | -33.8 (-64.7, -2.9)  | 0.032          | 0.294                         |
| Posterior                      | -10.3 (-29.6, 9.0)                 | 0.295          | 0.504                         | -4.2 (-26.6, 18.2)        | 0.714          | 0.800                         | -15.4 (-40.8, 10.1)       | 0.235          | 0.494                         | -9.7 (-30.1, 10.7)   | 0.348          | 0.526                         |
| Lateral ventricles             | 590.5 (-120.4, 1301)               | 0.103          | 0.361                         | 323.2 (-504.5, 1151)      | 0.441          | 0.561                         | 553.8 (-387.2, 1495)      | 0.247          | 0.494                         | 498.4 (-254.5, 1251) | 0.193          | 0.464                         |

Difference (95% CI) in brain structures adjusted for age at MRI, sex, intracranial volume, maternal education, residential neighborhood socioeconomic status, birthweight and mother's smoking status during pregnancy.

Abbreviations: PM<sub>2.5</sub>, Particle Matter with a diameter of 2.5  $\mu$ m or less.

† Raw p values not adjusted for multiple comparisons.

‡ False-discovery rate-corrected p values.

**Table 3. Difference (95% CI) in behavioral problems by corpus callosum volumes in 186 children.**

| Behavior                 | Corpus Callosum volumes, mm <sup>3</sup> |                |                               |                       |                |                               |                   |                |                               |
|--------------------------|--|----------------|-------------------------------|-----------------------|----------------|-------------------------------|-------------------|----------------|-------------------------------|
|                          | Total <sup>a</sup>                       |                |                               | Anterior <sup>b</sup> |                |                               | Body <sup>c</sup> |                |                               |
|                          | RR (95%CI)                               | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> | RR (95%CI)            | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> | RR (95%CI)        | p <sup>†</sup> | p <sub>FDR</sub> <sup>‡</sup> |
| ADHD score               |  |                |                               |                       |                |                               |                   |                |                               |
| Total*                   | 1.03 (0.96, 1.10)                        | 0.382          | 0.946                         | 1.00 (0.95, 1.06)     | 0.890          | 0.961                         | 1.04 (0.98, 1.11) | 0.171          | 0.684                         |
| Inattention**            | 1.01 (0.94, 1.08)                        | 0.812          | 0.961                         | 1.00 (0.95, 1.05)     | 0.917          | 0.961                         | 1.02 (0.96, 1.08) | 0.588          | 0.961                         |
| Hyperactivity **         | 1.07 (0.99, 1.16)                        | 0.085          | 0.510                         | 1.02 (0.96, 1.09)     | 0.472          | 0.946                         | 1.09 (1.01, 1.17) | 0.023          | 0.276                         |
| SDQ score                |  |                |                               |                       |                |                               |                   |                |                               |
| Total difficulties score | 1.00 (0.97, 1.03)                        | 0.961          | 0.961                         | 0.99 (0.97, 1.01)     | 0.473          | 0.946                         | 1.00 (0.98, 1.03) | 0.796          | 0.961                         |

Negative binomial regression models adjusted for age, sex and intracranial volume.

Abbreviations: ADHD, attention deficit hyperactivity disorder; SDQ, Strengths and Difficulties Questionnaire; RR, Rate Ratio.

\* n=181, \*\*n=182.

<sup>a</sup> by 100 mm<sup>3</sup> decrease.

<sup>b</sup> by 25 mm<sup>3</sup> decrease.

<sup>c</sup> by 50 mm<sup>3</sup> decrease.

† Raw p values not adjusted for multiple comparisons.

‡ False-discovery rate–corrected p values.