

Contents lists available at ScienceDirect

Environment International



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

Associations between air pollution and biomarkers of Alzheimer's disease in cognitively unimpaired individuals

Silvia Alemany^{a,b,c,1}, Marta Crous-Bou^{d,e,f,1,*}, Natalia Vilor-Tejedor^{b,d,g,h}, Marta Milà-Alomà^{b,d,i,j}, Marc Suárez-Calvet^{d,i,j,k}, Gemma Salvadó^{d,i}, Marta Cirach^{a,b,c}, Eider M. Arenaza-Urquijo^{d, i, j}, Gonzalo Sanchez-Benavides^{d, i, j}, Oriol Grau-Rivera^{d, i, j, k}, Carolina Minguillon^{d, i, j}, Karine Fauria^{d, i, j}, Gwendlyn Kollmorgen¹, Juan Domingo Gispert^{b,d,i,m}, Mireia Gascón^{a,b,c}, Mark Nieuwenhuijsen^{a,b,c}, Henrik Zetterberg^{n,o,p,q}, Kaj Blennow^{n,o}, Jordi Sunyer^{a,b,c,i}, José Luis Molinuevo^{d,*}, for the ALFA study²

^a ISGlobal, Barcelona Institute for Global Health - Campus MAR, Barcelona Biomedical Research Park. Barcelona, Spain

- ^b Universitat Pompeu Fabra (UPF). Barcelona, Spain
- Centro de Investigación Biomédica en Red de Epidemiología y Salud Pública (CIBERESP). Madrid, Spain
- ^d Barcelonaßeta Brain Research Center (BBRC), Pasqual Maragall Foundation, Barcelona, Spain
- e Unit of Nutrition and Cancer, Cancer Epidemiology Research Program, Catalan Institute of Oncology (ICO) Bellvitge Biomedical Research Institute (IDIBEL).
- L'Hospitalet de Llobregat, Barcelona, Spain
- ^f Department of Epidemiology, Harvard T.H. Chan School of Public Health. Boston, MA, USA
- ^g Centre for Genomic Regulation (CRG). The Barcelona Institute for Science and Technology, Barcelona, Spain
- ^h Erasmus MC. University Medical Center Rotterdam. Department of Clinical Genetics Center, Rotterdam, the Netherlands
- ⁱ IMIM Hospital del Mar Medical Research Institute. Barcelona, Spain
- ^j Centro de Investigación Biomédica en Red de Fragilidad y Envejecimiento Saludable (CIBER-FES). Madrid, Spain
- ^k Servei de Neurologia, Hospital del Mar, Barcelona, Spain
- ¹ Roche Diagnostics GmbH, Penzberg, Germany
- ^m Centro de Investigación Biomédica en Red de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Madrid, Spain
- ⁿ Department of Psychiatry and Neurochemistry, Institute of Neuroscience and Physiology, University of Gothenburg, Mölndal, Sweden
- ^o Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden
- ^p Department of Neurodegenerative Disease, UCL Institute of Neurology, Queen Square, London, United Kingdom
- ^q UK Dementia Research Institute at UCL, London, United Kingdom

ARTICLE INFO

Keywords:

Air pollution

AD biomarkers

Amyloid-β

APOE

ABSTRACT

Background: Air quality contributes to incidence of Alzheimer's disease (AD) although the underlying neurobiological mechanisms are unclear. This study was aimed to examine the association between air pollution and Nitrogen dioxide concentrations of cerebrospinal fluid (CSF) AD biomarkers and amyloid- β (A β) deposition. Particulate matter Participants and methods Neurodegeneration The sample included 156 cognitively unimpaired adults aged 57 years (61 at biomarkers assessment) with increased risk of AD from the ALFA + Study. We examined CSF levels of Aβ42, Aβ40, p-Tau, t-Tau, neurofilament light (NfL) and cerebral amyloid load (Centiloid). A Land Use Regression model from 2009 was used to estimate residential exposure to air pollutants including nitrogen dioxide (NO₂,) and particulate matter (PM_{2.5}, PM_{2.5} abs)

https://doi.org/10.1016/j.envint.2021.106864

Received 29 March 2021; Received in revised form 10 August 2021; Accepted 2 September 2021 Available online 16 September 2021

Abbreviations: AD, Alzheimer's dementia; Aβ, Amyloid- βeta; APOE-ε4, APOLIPOPROTEIN E (APOE) epsilon 4 (ε4) allele; β, βeta coefficient; ALFA, ALzheimer and FAmilies; CI, Confidence Interval; CSF, Cerebrospinal Fluid; CL, centiloid values; LUR, land-use regression models; MRI, Magnetic Resonance Imaging; NfL, neurofilament light; NO2, Nitrogen dioxide; NOx, Nitrogen Oxides; PET, positron emission tomography; p-tau, phosphorylated tau; PM, Particulate Matter; t-tau, total tau; SD, Standard Deviation; SE, Standard Error.

^{*} Corresponding authors at: Barcelonaßeta Brain Research Centre - Pasqual Maragall Foundation. C. Wellington, 30, 08005 Barcelona, Spain (M.C. Bou). Barcelonaßeta Brain Research Centre - Pasqual Maragall Foundation. C. Wellington, 30, 08005 Barcelona, Spain (J.L. Molinuevo).

E-mail addresses: mcrous@barcelonabeta.org (M. Crous-Bou), jlmolinuevo@barcelonabeta.org (J. Luis Molinuevo).

¹ Equal contributions.

 $^{^2}$ The complete list of collaborators of the ALFA Study can be found in the acknowledgements section.

^{0160-4120/© 2021} The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license eativecommons.org/licenses/by-nc-nd/4.0/).

 PM_{10}). This model was considered a surrogate of long-term exposure until time of data collection in 2013–2014. Participants have resided in the same residence for at least the previous 3 years. Multiple linear regression models were used to estimate associations between air pollutants and biomarkers. The effect modification by CSF A β status and *APOE-e4* carriership was also assessed.

Results: A consistent pattern of results indicated that greater exposure to NO₂ and PM_{2.5} absorbance was associated with higher levels of brain A β deposition, while greater exposure to PM₁₀ and PM_{2.5} was associated with higher levels of CSF NfL. Most associations were driven by individuals that were A β -positive. Although *APOE-e4* status did not significantly modify these associations, the effect of air pollutants exposure on CSF NfL levels was stronger in *APOE-e4* carriers.

Conclusion: In a population of cognitively unimpaired adults with increased risk of AD, long-term exposure to air pollution was associated with higher levels in biomarkers of AD pathology. While further research is granted to elucidate the mechanisms involved in such associations, our results reinforce the role of air pollution as an environmental risk factor for AD.

1. Introduction

Alzheimer's disease (AD) is a slowly progressive neurodegenerative disorder clinically characterised by deterioration of episodic memory and successive impairment of additional cognitive domains, with behavioural changes impacting activities of daily living. The pathological hallmarks of AD include the extracellular accumulation of amyloid- β (A β) and intracellular aggregates of hyperphosphorylated tau (Frisoni et al., 2017). Since the number of individuals affected by AD is expected to rise due to the aging population, and there is still no cure for this disease, there is increasing interest in identifying modifiable environmental risk factors (Crous-Bou et al., 2017).

Air pollution is an emerging environmental risk factor for AD but knowledge on the effect of this exposure on the central nervous system is limited. Air pollution exposure estimated using land-use regression models (LUR) has been associated with cognitive decline (Tzivian et al., 2016) and increased incidence for dementia, including AD, in elderly people (Oudin et al., 2016). In the light of these findings, a few epidemiological studies have considered the role of the *Apolipoprotein E* (*APOE*) epsilon 4 (ϵ 4) allele, the strongest known genetic risk factor for AD (Genin et al., 2011), as the link between air pollution and cognition and behaviour. Among children and older women from the general population, the adverse association between air pollution and cognition was stronger among ϵ 4 (Alemany et al., n.d.; Cacciottolo et al., 2020; Lilian Calderón-Garcidueñas et al., 2015; Schikowski et al., 2015).

Studies in *post-mortem* brain tissue provide biological support for the relationship between air pollution and AD. In autopsy samples from children and young adults who resided in high polluted areas, extracellular deposition of A β and phosphorylated tau (p-tau) were observed, which are considered hallmarks of AD (L. Calderón-Garcidueñas et al., 2018; Lilian Calderón-Garcidueñas, González-Maciel, et al., 2020). Interestingly, no evidence of AD neuropathological change was found among non-exposed individuals (Lilian Calderón-Garcidueñas et al., 2012). To date, only one study has investigated *in vivo* cerebrospinal fluid (CSF) biomarkers related to neurodegenerative disorders among urbanite children and clean air controls (Lilian Calderón-Garcidueñas et al., 2016). Thus, the extent to which different air pollutants can alter *in vivo* AD biomarkers such as A β and tau levels in humans, is yet to be established.

Furthermore, when examining potential associations between air pollution exposure and AD biomarkers, it is important to consider the role of APOE-c4 and the progression of the disease.

In the present study, we aimed to examine the association between air pollutants and CSF and positron emission tomography (PET) biomarkers related to brain A β deposition, tau pathology and neurodegeneration in cognitively unimpaired adult individuals at increased risk for developing AD. In addition, effect modification by *APOE-e4* and A β status in these associations was also investigated.

2. Methods

2.1. Study population

Participants were selected from the ALFA + study, a nested longitudinal long-term study of the ALFA (Alzheimer and Families) cohort (Molinuevo et al., 2016). The ALFA cohort was established as a research platform aimed to characterize preclinical AD among 2743 cognitively unimpaired individuals, aged between 45 and 75 years, and enriched for family history of AD. Participants were recruited between 2013 and 2014. Impaired cognition was determined by cognitive performance falling outside the established cutoffs of the following cognitive tests: Mini-Mental State Examination (Blesa et al., 2001; Folstein et al., 1975) (MMSE) ,26, or Memory Impairment Screen (Böhm et al., 2005; Buschke et al., 1999), 6, or Time-Orientation subtest of the Barcelona Test II (Quinones-Ubeda, 2009) (TO-BTII) ,68, or semantic fluency (Peña-Casanova et al., 2009; Ramier & Hécaen, 1970) (animals; SF), 12. The nested ALFA + study consists of a subset of participants of the ALFA parent cohort, who were invited to join based on their specific AD risk profile including AD parental history, APOE status and cognitive performance (Molinuevo et al., 2016). In the present study, we included the first consecutive 450 participants of ALFA + that were assessed between 2016 and 2019. From these, we selected only those individuals residing in the city of Barcelona in the same residence for at least the previous 3 years, and with reliable geocoded data leaving a final sample of 156 individuals. A flow chart depicting the final sample of the study is presented in Supplementary Fig. 1 (Fig S1).

The ALFA + study (ALFA-FPM-0311) was approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona, and registered at Clinicaltrials.gov (Identifier: NCT02485730). All participants signed the study's informed consent form that had also been approved by the Independent Ethics Committee "Parc de Salut Mar," Barcelona.

2.2. Exposure

Air pollution measurements were collected within the air pollution monitoring campaign of the European Study of Cohorts for Air Pollution Effects (ESCAPE; http://www.escapeproject.eu/) following established protocols (Beelen et al., 2013; Eeftens et al., 2012). Levels of air pollution exposure including nitrogen oxides (NO₂ and NO_x) and particulate matter (PM_{2.5} [particulate matter with aerodynamic diameter<2.5 µm], PM_{10} [<10 µm], PM coarse [$PM_{2.5-10}$, i.e., coarse particulate matter, between 2.5 µm and 10 µm], and PM2.5 absorbance [PM2.5 light absorption, which constitutes a marker of black or elemental carbon (Cyrys et al., 2003)] were measured in three different seasons (warm, cold, and one intermediate temperature season) to model an annual average for 2009. Given the high correlation between NO_2 and NO_x (r = 0.90), we present results only for NO₂. Exposures containing fine particles (PM_{2.5}, and PM_{2.5} absorbance) are particularly relevant given their ability to cross epithelial, endothelial, and neural barriers, producing direct and indirect brain damage (Block & Calderón-Garcidueñas, 2009). LUR

models were used to estimate individual levels of exposure to these air pollutants at the participant's residential addresses reported in 2013–2014 (this information was not available for 2009). The model for 2009 was considered as a surrogate of long-term exposure until the time of data collection (2013–2014) (Vert et al., 2017). The LUR model estimated in 2009 is considered representative of the posterior years because, even though air pollution levels have slightly decreased in Barcelona over the years (from year 2009 to the time of enrollment in the study in 2013–2014), spatial differences across the city have remained similar (i.e. subject's exposure globally decreased in the same proportion) (Vert et al., 2017). LUR models were based on geographical information system (GIS) and statistical methods.

2.3. CSF biomarker measurements

We measured CSF levels of A β 42, A β 40, phosphorylated tau (p-tau), total tau (t-tau) and neurofilament light (NfL). The CSF A_β42/40 ratio was used as a biomarker of A β pathology, and the cutoff for A β positivity (CSF A β 42/40 < 0.071) was determined by a Gaussian mixture model as previously described (Milà-Alomà et al., 2020). The collection of CSF biomarkers in ALFA + is described elsewhere (Milà-Alomà et al., 2019; Milà-Alomà et al., 2020). In brief, CSF t-tau and p-tau were measured using the electrochemiluminiscence immunoassays Elecsys® on a fully automated cobas e 601 instrument (Roche Diagnostics, Rotkreuz, Switzerland). CSF A_{β42}, A_{β40} and NfL were measured with the prototype NeuroToolKit on a cobas e 411 or e 601 instrument (Roche Diagnostics, Rotkreuz, Switzerland). All measurements were performed at the Clinical Neurochemistry Laboratory, Sahlgrenska University Hospital, Mölndal, Sweden. All measures of CSF AD biomarkers were treated as continuous variables where higher levels indicate more pathological levels except for the CSF $A\beta 42/40$ ratio, where lower values indicate more pathology.

2.4. Positron emission tomography (PET) scanning

Levels of brain A β deposition were assessed by PET. A T1-weighted MRI and an [¹⁸F]flutemetamol PET scan were acquired in all participants (mean time difference 97.1 days; range [14–343]). The T1-weighted 3D-TFE sequence was acquired in a Philips 3 T Ingenia CX scanner with a voxel size of $0.75 \times 0.75 \times 0.75$ mm3, FOV $240 \times 240 \times 180$ mm3, sagittal acquisition, flip angle 8°, TR = 9.9 ms, TE = 4.6 ms, TI = 900 ms. PET imaging was conducted in a Siemens Biograph mCT, following a cranial CT scan for attenuation correction. Participants were injected with 185 MBq (range 166.5–203.5 Mbq) of [¹⁸F]flutemetamol, and 4 frames of 5 min each were acquired 90 min post-injection. Images

were reconstructed with an OSEM3D algorithm using 8 iterations and 21 subsets and with point spread function (PSF) and time of flight (TOF) corrections into a matrix size of $1.02 \times 1.02 \times 2.03$ mm.

All PET images were pre-processed using a validated Centiloid pipeline (Klunk et al., 2015) using SPM12 (https://www.fil.ion.ucl.ac. uk/spm/software/spm12/). PET frames were realigned, averaged and co-registered to their corresponding MRI scans. MRIs were then normalized to the MNI space together with PET images. We calculated the standardized uptake value ratio (SUVr) in MNI space using the target region provided in the GAAIN website (<u>http://www.gaain.org/centiloid-project</u>) and the whole cerebellum as reference region. SUVr values were then transformed to the Centiloid scale as detailed elsewhere (Salvadó et al., 2019). Centiloid values (CL) as a measure of brain A β deposition were treated as continuous variable where higher levels indicate higher A β burden.

2.5. Statistical analyses

Multiple linear regression analyses were conducted to examine whether levels of air pollutants (i.e. NO2, PM2.5, PM10 and PM2.5 absorbance) were associated with AD biomarkers (i.e. AB42/40 ratio, ptau, t-tau, NfL and CL as a measure of brain $A\beta$ deposition). In these models, biomarkers were assigned as dependent variables and air pollutants as independent variables. Each pollutant was tested for association with each biomarker individually. We excluded the extreme values of the outcome variables (i.e. biomarkers) defined as either those values that fell outside of 3 times the interquartile range below the first quartile (Q1) or 3 times the interquartile range above the third quartile (Q3). Levels of p-tau, t-tau and NfL were log10-transformed for normality. All models were adjusted by sex, age at recruitment and APOE-e4 status (defined as carrier of at least one APOE-ɛ4 allele vs. non-carriers). Age at recruitment was strongly correlated with age at biomarker assessment (r = 0.98, p = 2.2-e16). Standardized regression coefficients are reported through results (β). Standardized coefficients represent the mean change in the outcome (measurement unit is pg/mL) given a one standard deviation change in the exposure (measurement unit is $\mu g/m^3$).

In secondary analyses, we examined the potential effect modification by CSF A β status and *APOE-e4* carriership. We conducted sensitivity analysis further adjusting by lifestyle and clinical risk factors for AD including years of education, physical activity, body mass index (BMI), smoking habits and family history of AD.

Statistical significance was set at P < 0.05. All analyses were conducted using R statistical software package, version 3.6.2 (http: <u>www.r-project.org/</u>).



Fig. 1. Dot-and-whisker plot showing associations between exposure to air pollutants and levels of Alzheimer's disease (AD) biomarkers. All estimates were adjusted by age, age and *APOE*- ϵ 4 status. Standardized coefficients and 95% confidence intervals are reported. Note: NfL, neurofilament light; CL, centiloid values.

3. Results

The main characteristics of the study population are shown in Table 1. Mean age of participants was 56.7 years at recruitment (SD = 5.2), 60.6 (SD = 4.9) at CSF extraction and 60.9 (SD = 4.9) at PET scan. Sex was equally distributed (55.8% were female), 48.7% were carriers of the *APOE*- ϵ 4 allele, and 60.9% had a relative with dementia. Socio-demographic characteristics of the study population according to A β and *APOE*- ϵ 4 status are described in Supplementary Table 1 (Table S1) and Table S2, respectively.

3.1. Association between air pollutants and AD biomarkers

Associations between exposure to air pollutants and AD biomarkers are shown in Fig. 1 and Table S3. Higher exposure to NO₂ was associated with higher CL (NO₂: $\beta = 0.21$, 95% CI = 0.05–0.37, P = 0.012). Positive associations were observed between exposure to PM_{2.5} and PM₁₀, and CSF NfL levels (PM_{2.5}: $\beta = 0.17$, 95% CI = 0.02–0.31, P = 0.027; PM₁₀: β = 0.15, 95% CI = 0.01–0.30, P = 0.047). Additionally, exposure to PM_{2.5} absorbance was associated with centiloid scale ($\beta = 0.16$, 95% CI = 0–2-0.32, P = 0.050).

The direction of effects was consistently positive for all biomarkers

Table 1

Main characteristics of the study population (N = 156). Missing data is indicated, otherwise, data was available for all individuals.

	Study population
Age at recruitment (years) (mean, SD)	56.7 (5.2)
Age at biomarkers assessment (years) (mean, SD)	60.6 (4.9)
Sex (N, %)	
Female	87 (55.8)
Male	69 (44.2)
Years of education (mean, SD)	14.4 (3.3)
Family history of AD ^a (N, %)	
No	4 (2.6)
After 75 years	57 (36.5)
Before 75 years	95 (60.9)
Smoking status (N, %)	
Current	38 (24.4)
Former	85 (54.5)
Never	24 (15.4)
BMI (mean, SD)	26.6 (4.1)
Physical activity ^b (mean, SD)	2769.9 (3293.7)
APOE status (N, %)	
ε4 carriers	76 (48.7)
ε4 non-carriers	80 (51.3)
A β status (N, %)	
Aβ-Positive	45 (30.6)
Aβ-Negative	102 (69.4)
AD biomarkers-CSF (mean, SD)	
Aβ42/40 ratio	0.1 (0.0)
Missing (N, %)	8 (5.1)
p-tau (pg/mL)	15.4 (5.8)
Missing (N, %)	8 (5.1)
t-tau (pg/mL)	192.2 (65.2)
Missing (N, %)	8 (5.1)
NfL (pg/mL)	81.3 (28.7)
Missing (N, %)	14 (8.9)
AD biomarkers- Aβ PET (mean, SD)	
CL ^c	2.0 (17.8)
Missing (N, %)	25 (16.0)
Air pollutants (mean, SD)	
$NO_2 (\mu g/m^3)$	57.6 (10.9)
$PM_{2.5} (\mu g/m^3)$	17.3 (2.2)
PM₁₀ (μg/m ³)	37.9 (4.1)
PM_{2.5} absorbance (µg/m ³)	2.8 (0.6)

NOTE: AD, Alzheimer's Disease; CSF, cerebrospinal fluid; NfL, neurofilament light; PET, positron emission tomography.

^a Having at least one parent with dementia regardless of age of onset.

^b Metabolic equivalents (MET_S) per week.

 $^{c}\,$ CL: Centiloid values as a measure of brain A β deposition. Whole cerebellum as reference region. Range: $-17.7,\,81.6.$

except for the CSF A β 42/40 ratio suggesting that air pollution was related to more pathological levels of all AD biomarkers analysed. (Fig. 1).

In sensitivity analyses further adjusting by lifestyle and risk factors of AD (i.e. family history of AD, years of education, BMI, smoking habits, and physical activity), the observed associations between air pollutants and AD biomarkers were slightly attenuated (Table S4). The associations between NO₂, PM_{2.5} absorbance and CL remained significant. Although the associations between PM_{2.5} and PM₁₀ and CSF NfL were no longer significant, we observed the same direction of effects and estimates of similar magnitude.

3.2. Effect modification $A\beta$ status

Associations between exposure to air pollutants and AD biomarkers stratified by the CSF A β 42/40 ratio (A β status) are shown in Fig. 2. Forty-five individuals were $A\beta$ positive and 102 were $A\beta$ negative. $A\beta$ status modified associations between air pollutants (NO2, PM2.5, PM10 and PM2.5 absorbance) and AD biomarkers (CSF p-tau, t-tau and NfL and CL) (*P* for interaction < 0.05). Only among individuals with a positive A β status, exposure to NO₂ was positively associated with CSF t-tau (β = 0.34, 95% CI = 0.04–0.64, P = 0.027), CSF NfL ($\beta = 0.36$, 95% CI = 0.12–0.60, P = 0.005) and CL ($\beta = 0.36$, 95% CI = 0.08–0.64, P =0.012). Similarly, associations between PM2.5, PM10 and PM2.5 absorbance and CSF p-tau, t-tau and CSF NfL were consistently positive and limited to $A\beta$ -positive participants (Fig. 2). When further adjusting by APOE-e4 status, associations between NO₂, PM_{2.5}, PM₁₀ and PM_{2.5} absorbance and CL remained positive and stronger among CSF Aβ-positive individuals to negatives (*P*-interaction < 0.05). Although we did not observe other significant interactions, positive associations between NO2, PM2.5, PM10 and PM2.5 absorbance and CSF t-tau and NfL were limited to the A β -positive group (Table S5).

3.3. Effect modification by APOE- ε 4 status

Associations between exposure to air pollutants and AD biomarkers stratified by *APOE-*ε4 status are shown in Table 4. Seventy-six individuals were APOE-ε4 carriers and 80 were non-carriers. Associations were not significantly different between carriers and non-carriers of the ε4 allele of the *APOE* (*P*-interaction > 0.05). Stratified analysis showed that several air pollutants were associated with CSF NfL (NO₂: $\beta = 0.25$, 95% CI = 0.03–0.47, *P* = 0.025; PM₁₀: $\beta = 0.25$, 95% CI = 0.04–0.46, *P* = 0.023; PM_{2.5} absorbance: $\beta = 0.24$, 95% CI = 0.01–0.36, *P* = 0.042) and CSF t-tau (PM_{2.5} absorbance: $\beta = 0.24$, 95% CI = 0.01–0.48, *P* = 0.045) levels only among carriers (N = 76). In contrast, NO₂ ($\beta = 0.26$, 95% CI = 0.02–0.49, *P* = 0.034), PM_{2.5} ($\beta = 0.25$, 95% CI = 0.03–0.47, *P* = 0.028) and PM_{2.5} absorbance ($\beta = 0.31$, 95% CI = 0.08–0.54, *P* = 0.009) showed associations with CL only among non-carriers (N = 80) (Fig. 3).

4. Discussion

In a population of late/middle-aged cognitively unimpaired adults with increased risk of AD, long-term air pollution exposure showed associations with biomarkers of AD pathology assessed in CSF and A β PET. The pattern of results was consistent suggesting detrimental effects of air pollution on AD risk. Specifically, greater exposure to NO₂ and PM_{2.5} absorbance was associated with higher levels of brain A β deposition (as assessed by A β PET), while PM₁₀ and PM_{2.5} exposure was positively associated with higher levels of CSF NfL, a marker of neuronal injury. Furthermore, in participants within the Alzheimer's *continuum*, defined by decreased CSF A β 42/40 ratio (positive A β status), greater exposure to NO₂, PM₁₀ and PM_{2.5} was related with greater tau-related biomarkers and NfL and NO₂ with tau pathology. Although *APOE-e4* status did not significantly modify these associations, the effect of air pollutants exposure on CSF NfL levels was stronger in *APOE-e4* carriers.



Fig. 2. Association between exposure to air pollution and levels of Alzheimer's disease (AD) biomarkers by amyloid status. Each point depicts the value of the AD biomarker of an individual and the solid lines indicate the regression line for each of the groups (positive (A +) and negative (A-) amyloid status. P-value for the interaction between air pollution and amyloid status is shown in the upper right corner of each plot. Results for stratified analysis are reported below each plot. All estimates were adjusted by sex, age and *APOE*- ϵ 4 status. Standardized coefficients (β) are reported. Note: NfL, neurofilament light; CL, centiloid values.

Additionally, the associations between exposure to air pollutants and CL were stronger among non-carriers.

To our knowledge, this is the first study analysing associations between estimated exposure to air pollutants and *in vivo* AD biomarkers assessed in CSF and A β PET in humans. These findings are biologically plausible given that evidence suggests that fine particles can reach the brain via the circulation, bypassing the blood–brain-barrier by direct translocation through the olfactory bulb (Oberdörster et al., 2004; Peters et al., 2006). Also, air pollution is among the most relevant sources of environmentally induced inflammation and oxidative stress, both involved in neurodegenerative processes (Block & Calderón-Garcidueñas, 2009; Brockmeyer & D'Angiulli, 2016).

Our results are in line with previous animal and human autopsy studies suggesting that air pollution may be adversely associated with characteristics of A β and tau-pathology (M Cacciottolo et al., 2017; Calderon-Garciduenas et al., 2003; Lilian Calderón-Garcidueñas et al., 2002, 2012; Lilian Calderón-Garcidueñas, Herrera-Soto, et al., 2020; Park et al., 2020). Air pollution has also been linked to brain structural alterations of relevance for AD such as progressive atrophy of gray and white matter (de Prado Bert et al., 2018). A recent study found that increased neuroanatomic risk for AD (a structural brain MRI-based score reflecting high-dimensional grey matter atrophies in brain areas vulnerable to Alzheimer's disease neuropathology) mediated the association between long-term exposure to ambient PM2.5 at residential

locations and declines in episodic memory (Younan et al., 2020). In this context, a previous study within the ALFA cohort showed that air pollution was associated with global atrophy and reduced volume and thickness in AD vulnerable regions (Crous-Bou et al., 2020). Our findings may be underlying these associations and overall provide support for the link between air pollution and AD-related outcomes.

Interestingly, we observed that participants within the Alzheimer's continuum (Sperling et al., 2013), hence already A β -positive, drove the associations with $A\beta$, tau and neurodegeneration biomarkers. AD has an extensive preclinical stage, which may be initiated 15 to 20 years before the emergence of clinical symptoms (Sperling et al., 2013). Aβ negativity, especially when defined with A^β CSF biomarkers, may indicate that AD-pathology is not yet present (Palmqvist et al., 2016). These results suggest that the detrimental effects linked to air pollution occurred downstream to Ap pathology. Air pollution will therefore contribute to the tau and neurodegenerative changes downstream to $A\beta$ pathology, hence affecting only those that are already within the continuum. A possible explanation for this finding is that $A\beta$ -positive individuals may be more vulnerable to air pollution effects. In this regard, it is worth mentioning that a high proportion of Aβ-positive individuals (76%) were also APOE- ε 4 carriers which may be accounting for their A β status and contributing to a greater vulnerability to air pollution. In future follow-ups, it will be relevant to examine whether associations between air pollution and AD-biomarkers observed at this time-point



Fig. 3. Association between exposure to air pollution and levels of Alzheimer's disease (AD) biomarkers by *APOE*- ϵ 4 status. Each point depicts the value of the AD biomarker of an individual and the solid lines indicate the regression line for each of the groups (carriers and non-carriers of the *APOE*- ϵ 4 allele). P-value for the interaction between air pollution and amyloid status is shown in the upper right corner of each plot. Results for stratified analysis are reported below each plot. All estimates were adjusted by sex and age. Standardized coefficients (β) are reported. Note: NfL, neurofilament light; CL, centiloid values.

change as AD-pathology evolves in some individuals.

The lack of associations between air pollutants and brain Aß deposition among carriers may be explained by the role of APOE in AB metabolism (Liu et al., 2013). APOE is related to the formation and aggregation of Aβ plaques (Kanekiyo et al., 2014), being Aβ deposition more prevalent among APOE-e4 carriers compared to non-carriers (Reiman et al., 2009; Snellman et al., 2020). Thus, variability in biomarkers related to $A\beta$ deposition could be mostly accounted for by APOE-e4 allele while among non-carriers, other risk factors such as air pollution could be triggering or contributing to these pathological characteristics. This highlights the need to identify modifiable risk factors for AD. It is also relevant to consider the temporal progression of AD-biomarkers to interpret these findings. CSF $A\beta$ alterations are amongst the first detectable pathophysiological events in AD while characteristics of tau pathology and neurodegeneration are thought to appear later (Clifford et al., 2016; Palmqvist et al., 2016). Exposure to air pollutants showed positive associations with CSF NfL and t-tau levels only in carriers, where pathology may be more advanced. Alternatively, carrying the $\varepsilon 4$ allele may also contribute to a greater vulnerability to other risk factors, either genetic or environmental, which exacerbate or accelerate AD-pathology. Indeed, findings in mice showed that APOE- $\varepsilon 4$ carriers had more PM-induced Aβ- plaques compared to non-carriers (M Cacciottolo et al., 2017). Although we did not observe associations between air pollution and centiloid scale among APOE-e4 carriers, associations with CSF t-tau and NfL were only present in this subgroup.

The current study must be interpreted in the context of its limitations. First, the sample was selected to include individuals at risk for AD, thus, although individuals were cognitively unimpaired, generalizations to the general population should be conducted with caution. For instance, \approx 50% of the individuals were carriers of the *APOE-e4* allele while its frequency in the general population ranges between 10% and 20% (Singh et al., 2006). Nevertheless, analyses were adjusted by *APOE-e4* status. Second, air pollution exposure estimations were based on participants' home address. Data on exposure to air pollution related to mobility or workplace is not available, thus, we cannot discard exposure misclassification. Third, air pollution exposure based on 2009 models was used as a surrogate of the period of data collection, which took place 4–10 years later. Nevertheless, as above mentioned, there is evidence indicating that the spatial distribution of air pollution in Barcelona has remained overall consistent in the past 20 years (Cesaroni et al., 2012; Wang et al., 2013). Fourth, due to the limited sample size of our study, rather than adjusting for multiple comparisons, we emphasized on the pattern and consistency of results.

Considering the consistent pattern of results observed, we believe that our findings reinforce the emerging role of air pollution as a major environmental risk factor for AD. The association between air pollutants and AD-biomarkers suggest that this exposure may contribute to the etiopathogenesis of AD. Future analyses in ALFA + will allow testing whether air pollution effects are related to the progression of the disease. Given the ubiquitous nature of this exposure, even if associated risk is of small effect size, reductions of population-level exposure would lead to decrease the global burden of the disease.

Author contributions

SA, MCB, JS and JLM were responsible for the initial plan, study design, conducting the study, and data interpretation. SA and MCB are responsible of statistical analysis, data interpretation and manuscript drafting. CM and KF were responsible for recruitment, data collection and data depuration. MMA, MSC, GS, GK, HZ, KB were responsible for biomarkers data acquisition and preparation. NVT was responsible for genetic data acquisition and preparation. MC, MG and MN were responsible of exposure data estimation. All authors interpreted the results, and critically revised the paper. SA, MCB, JS and JLM are guarantors and had full access to all the data, including statistical reports and tables, and take full responsibility for the integrity of the data and the accuracy of the data analysis. The corresponding authors (MCB, JLM) attest that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted.

CRediT authorship contribution statement

Silvia Alemany: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, writing-review & editing. Marta Crous-Bou: Conceptualization, Methodology, Formal analysis, Visualization, Writing-original draft, Writing-review & editing, Supervision. Natalia Vilor-Tejedor: Data curation, Methodology, Visualization, Writing-review & editing. Marta Milà-Alomà, Marc Suárez-Calvet, Gemma Salvadó, Marta Cirach, Eider M.Arenaza-Urquijo, Gonzalo Sanchez-Benavides, Oriol Grau Rivera, , Gwendlyn Kollmorgen , Henrik Zetterberg, Kaj Blennow: Data curation,Writing-review & editing. Carolina Minguillon, Karine Fauria: Data curation,Project administration. Juan Domingo Gispert, Mireia Gascón, Mark Nieuwenhuijsen: Methodology, Resources, Writing-review & editing. Jordi Sunyer: Supervision, Writing-review & editing. José Luis Molinuevo: Funding acquisition, Supervision, Writing-review & editing.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: [HZ has served at scientific advisory boards for Denali, Roche Diagnostics, Wave, Samumed, Siemens Healthineers, Pinteon Therapeutics and CogRx, has given lectures in symposia sponsored by Fujirebio, Alzecure and Biogen, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program (outside submitted work).

KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program. KB has served as a consultant, at advisory boards, or at data monitoring committees for Abcam, Axon, Biogen, JOMDD/Shimadzu. Julius Clinical, Lilly, MagQu, Novartis, Roche Diagnostics, and Siemens Healthineers, and is a co-founder of Brain Biomarker Solutions in Gothenburg AB (BBS), which is a part of the GU Ventures Incubator Program.

GK is a full-time employee of Roche Diagnostics GmbH.

JLM is currently a full-time employee of Lundbeck and has before served as a consultant or at advisory boards for the following for-profit companies, or has given lectures in symposia sponsored by the following for-profit companies: Roche Diagnostics, Genentech, Novartis, Lundbeck, Oryzon, Biogen, Lilly, Janssen, Green Valley, MSD, Eisai, Alector, BioCross, GE Healthcare, ProMIS Neurosciences, NovoNordisk, Zambón, Cytox and Nutricia.

The rest of the authors have no conflict of interest to declare.

Acknowledgements

This publication is part of the ALFA study (ALzheimer and FAmilies). The authors would like to express their most sincere gratitude to the ALFA project participants and relatives without whom this research would have not been possible.

Collaborators of the ALFA study are: Annabella Beteta, Raffaele Cacciaglia, Alba Cañas, Carme Deulofeu, Maria Emilio, Irene Cumplido, Ruth Dominguez, Carles Falcon, Sherezade Fuentes, Laura Hernandez, Gema Huesa, Jordi Huguet, Paula Marne, Tania Menchón, Grégory Operto, Albina Polo, Sandra Pradas, Aleix Sala-Vila, Mahnaz Shekari, Anna Soteras, and Marc Vilanova.

The authors thank Roche Diagnostics International Ltd. for providing the kits to measure CSF biomarkers and GE Healthcare for kindly providing [18 F]flutemetamol doses of ALFA + participants. ELECSYS, COBAS, and COBAS E are registered trademarks of Roche.

Funding sources

The project leading to these results has received funding from "la Caixa" Foundation (ID 100010434), under agreement LCF/PR/GN17/50300004 and the Alzheimer's Association and an international anonymous charity foundation through the TriBEKa Imaging Platform project (TriBEKa-17-519007). Additional support has been received from the Universities and Research Secretariat, Ministry of Business and Knowledge of the Catalan Government under the grant no. 2017-SGR-892.

SA is funded by a Juan de la Cierva - Incorporación Postdoctoral Contract awarded by Ministry of Economy, Industry and Competitiveness (IJCI-2017-34068). NV-T is funded by a post-doctoral grant, Juan de la Cierva Programme (FJC2018-038085-I), Ministry of Science and Innovation- Spanish State Research Agency. MS-C received funding from the European Union's Horizon 2020 Research and Innovation Program under the Marie Sklodowska-Curie action grant agreement No 752310, and currently receives funding from Instituto de Salud Carlos III (PI19/00155) and from the Spanish Ministry of Science, Innovation and Universities (Juan de la Cierva Programme grant IJC2018-037478-I). EMA-U is supported by the Spanish Ministry of Science, Innovation and Universities - Spanish State Research Agency (RYC2018-026053-I). OG-R is supported by the Spanish Ministry of Science, Innovation and Universities (FJCI-2017-33437). JDG is supported by the Spanish Ministry of Science and Innovation (RYC-2013-13054). HZ is a Wallenberg Scholar supported by grants from the Swedish Research Council (#2018-02532), the European Research Council (#681712), Swedish State Support for Clinical Research (#ALFGBG-720931), the Alzheimer Drug Discovery Foundation (ADDF), USA (#201809-2016862), and the UK Dementia Research Institute at UCL.

KB is supported by the Swedish Research Council (#2017-00915), the Alzheimer Drug Discovery Foundation (ADDF), USA (#RDAPB-201809-2016615), the Swedish Alzheimer Foundation (#AF-742881), Hjärnfonden, Sweden (#FO2017-0243), the Swedish state under the agreement between the Swedish government and the County Councils, the ALF-agreement (#ALFGBG-715986), and European Union Joint Program for Neurodegenerative Disorders (JPND2019-466-236). All CRG authors acknowledge the support of the Spanish Ministry of Science, Innovation and Universities to the EMBL partnership, the Centro de Excelencia Severo Ochoa and the CERCA Programme/Generalitat de Catalunya. ISGlobal acknowledge support from the Spanish Ministry of Science and Innovation through the "Centro de Excelencia Severo Ochoa 2019–2023" Program (CEX2018-000806-S), and support from the Generalitat de Catalunya through the CERCA Program

Data statement

The data that support the findings of this study are available from the corresponding authors (MCB, JLM), upon reasonable request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2021.106864.

References

- Alemany, S., Vilor-Tejedor, Natàlia García-Esteban, Raquel Bustamante, M., Dadvand, P., Esnaola, M., Mortamais, M., Forns, J., van Drooge, B. L., Alvárez-Pedrerol, M., Grimalt, J., Rivas, I., Querol, X., Pujol, J., & Sunyer, J. (n.d.). Traffic air pollution, APOE e4 status and neurodevelopmental outcomes in scholar children.
- Beelen, R., Hoek, G., Vienneau, D., Eeftens, M., Dimakopoulou, K., Pedeli, X., Tsai, M.-Y., Künzli, N., Schikowski, T., Marcon, A., Eriksen, K.T., Raaschou-Nielsen, O., Stephanou, E., Patelarou, E., Lanki, T., Yli-Tuomi, T., Declercq, C., Falq, G., Stempfelet, M., Birk, M., Cyrys, J., von Klot, S., Nádor, G., Varró, M.J., Dédelé, A., Gražulevičiene, R., Mölter, A., Lindley, S., Madsen, C., Cesaroni, G., Ranzi, A., Badaloni, C., Hoffmann, B., Nonnemacher, M., Krämer, U., Kuhlbusch, T., Cirach, M., de Nazelle, A., Nieuwenhuijsen, M., Bellander, T., Korek, M., Olsson, D.,

Environment International 157 (2021) 106864

Strömgren, M., Dons, E., Jerrett, M., Fischer, P., Wang, M., Brunekreef, B., de Hoogh, K., 2013. Development of NO2 and NOx land use regression models for estimating air pollution exposure in 36 study areas in Europe – The ESCAPE project. Atmos. Environ. 72, 10–23. https://doi.org/10.1016/j.atmosenv.2013.02.037.

- Blesa, R., Pujol, M., Aguilar, M., Santacruz, P., Bertran-Serra, I., Hernández, G., Sol, J.M., Peña-Casanova, J., Soler, T., Zabay, C., Riera, M., Castellví, M., Torner, L., Charques, I., Toirán, H., Manero, R.M., Peter Böhm, G.E., Martí, A.M., Meza, M., Crespo, M.C., 2001. Clinical validity of the "mini-mental state" for Spanish speaking communities. Neuropsychologia 39 (11), 1150–1157. https://doi.org/10.1016/ S0028-3932(01)00055-0.
- Block, M.L., Calderón-Garcidueñas, L., 2009. Air Pollution: Mechanisms of Neuroinflammation & CNS Disease. Trends Neurosci 32 (9), 506–516. https://doi. org/10.1016/j.tins.2009.05.009.Air.
- Böhm, P., Peña-Casanova, J., Gramunt, N., Manero, R.M., Terrón, C., Quiñones-Ubeda, S., 2005. Spanish version of the Memory Impairment Screen (MIS): normative data and discriminant validity. Neurologia (Barcelona, Spain) 20 (8), 402–411. https://pubmed.ncbi.nlm.nih.gov/16217689/.
- Brockmeyer, S., D'Angiulli, A., 2016. How air pollution alters brain development: the role of neuroinflammation. Translational Neuroscience 7, 24–30. https://doi.org/ 10.1515/tnsci-2016-0005.
- Buschke, H., Kuslansky, G., Katz, M., Stewart, W.F., Sliwinski, M.J., Eckholdt, H.M., Lipton, R.B., 1999. Screening for dementia with the Memory Impairment Screen. Neurology 52 (2), 231–238. https://doi.org/10.1212/wnl.52.2.231.
- Cacciottolo, M, Wang, X., Driscoll, I., Woodward, N., Saffari, A., Reyes, J., Serre, M. L., Vizuete, W., Sioutas, C., Morgan, T. E., Gatz, M., Chui, H. C., Shumaker, S. A., Resnick, S. M., Espeland, M. A., Finch, C. E., & Chen, J. C. (2017). Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. Translational Psychiatry, 7 (1), e1022. DOI: 10.1038/tp.2016.280.
- Cacciottolo, M., Morgan, T.E., Saffari, A.A., Shirmohammadi, F., Forman, H.J., Sioutas, C., Finch, C.E., 2020. Traffic-related air pollutants (TRAP-PM) promote neuronal amyloidogenesis through oxidative damage to lipid rafts. Free Radical Biol. Med. 147, 242–251. https://doi.org/10.1016/j.freeradbiomed.2019.12.023.
- Calderón-Garcidueñas, L., Gónzalez-Maciel, A., Reynoso-Robles, R., Delgado-Chávez, R., Mukherjee, P.S., Kulesza, R.J., Torres-Jardón, R., Ávila-Ramírez, J., Villarreal-Ríos, R., 2018. Hallmarks of Alzheimer disease are evolving relentlessly in Metropolitan Mexico City infants, children and young adults. APOE4 carriers have higher suicide risk and higher odds of reaching NFT stage V at ≤ 40 years of age. Environ. Res. 164, 475–487. https://doi.org/10.1016/J.ENVRES.2018.03.023.
- Calderon-Garciduenas, L., Maronpot, R.R., Torres-Jardon, R., Henriquez-Roldan, C., Schoonhoven, R., Acuna-Ayala, H., Villarreal-Calderon, A., Nakamura, J., Fernando, R., Reed, W., Azzarelli, B., Swenberg, J.A., 2003. DNA Damage in Nasal and Brain Tissues of Canines Exposed to Air Pollutants Is Associated with Evidence of Chronic Brain Inflammation and Neurodegeneration. Toxicol. Pathol. 31 (5), 524–538. https://doi.org/10.1080/01926230390226645.
- Calderón-Garcidueñas, L., Avila-Ramírez, J., Calderón-Garcidueñas, A., González-Heredia, T., Acuña-Ayala, H., Chao, C.K., Thompson, C., Ruiz-Ramos, R., Cortés-González, V., Martínez-Martínez, L., García-Pérez, M.A., Reis, J., Mukherjee, P.S., Torres-Jardón, R., Lachmann, I., 2016. Cerebrospinal Fluid Biomarkers in Highly Exposed PM2.5 Urbanites: The Risk of Alzheimer's and Parkinson's Diseases in Young Mexico City Residents. J. Alzheimer's Disease 54 (2), 597–613. https://doi. org/10.3233/JAD-160472.
- Calderón-Garcidueñas, Lilian, Azzarelli, B., Acuna, H., Garcia, R., Gambling, T. M., Osnaya, N., Monroy, S., Tizapantzi, M. D. R., Carson, J. L., Villarreal-Calderon, A., & Rewcastle, B. (2002). Air pollution and brain damage. Toxicologic Pathology, 30(3), 373–389. https://doi.org/10.1080/01926230252929954.
- Calderón-Garcidueñas, L., Kavanaugh, M., Block, M., D'Angiulli, A., Delgado-Chávez, R., Torres-Jardón, R., González-Maciel, A., Reynoso-Robles, R., Osnaya, N., Villarreal-Calderon, R., Guo, R., Hua, Z., Zhu, H., Perry, G., Diaz, P., 2012. Neuroinflammation, hyperphosphorylated tau, diffuse amyloid plaques, and down-regulation of the cellular prion protein in air pollution exposed children and young adults. J. Alzheimer's Disease 28 (1), 93–107. https://doi.org/10.3233/JAD-2011-110722.
- Calderón-Garcidueñas, L., Mora-Tiscareño, A., Franco-Lira, M., Zhu, H., Lu, Z., Solorio, E., Torres-Jardón, R., D'Angiulli, A., 2015. Decreases in Short Term Memory, IQ, and Altered Brain Metabolic Ratios in Urban Apolipoprotein e4 Children Exposed to Air Pollution. J. Alzheimer's Disease 45 (3), 757–770. https:// doi.org/10.3233/JAD-142685.
- Cesaroni, G., Porta, D., Badaloni, C., Stafoggia, M., Eeftens, M., Meliefste, K., Forastiere, F., 2012. Nitrogen dioxide levels estimated from land use regression models several years apart and association with mortality in a large cohort study. Environ. Health: A Global Access Science Source 11 (1). https://doi.org/10.1186/ 1476-069X-11-48.
- Clifford Jr., R.J., Knopman, D.S., Jagust, W.J., Petersen, R.C., Weiner, M.W., Aisen, P.S., Shaw, L.M., Vemuri, P., Wiste, H.J., Weigand, S.D., Lesnick, T.G., Pankratz, V.S., Donohue, M.C., Trojanowski, J.Q., Zlokovic, B.V., Morris, A.W.J., Carare, R.O., Schreiber, S., Hawkes, C.A., Malm, J., 2016. Update on hypothetical model of Alzheimer's disease biomarkers. Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism 12 (2), 207–216. https://doi.org/10.1177/0271678X16629486.
- Crous-Bou, M., Gascon, M., Gispert, J.D., Cirach, M., Sánchez-Benavides, G., Falcon, C., Arenaza-Urquijo, E.M., Gotsens, X., Fauria, K., Sunyer, J., Nieuwenhuijsen, M.J., Luis Molinuevo, J., 2020. Impact of urban environmental exposures on cognitive performance and brain structure of healthy individuals at risk for Alzheimer's dementia. Environ. Int. 138, 105546. https://doi.org/10.1016/j. envint.2020.105546.

- Crous-Bou, M., Minguillón, C., Gramunt, N., & Molinuevo, J. L. (2017). Alzheimer's disease prevention: From risk factors to early intervention. In Alzheimer's Research and Therapy (Vol. 9, Issue 1, p. 71). BioMed Central Ltd. DOI: 10.1186/s13195-017-0297-z.
- Cyrys, J., Heinrich, J., Hoek, G., Meliefste, K., Lewné, M., Gehring, U., Bellander, T., Fischer, P., Vliet, P. van, Brauer, M., Wichmann, H.-E., & Brunekreef, B. (2003). Comparison between different traffic-related particle indicators: Elemental carbon (EC), PM2.5 mass, and absorbance. J. Exposure Sci. Environ. Epidemiol. 2003 13:2, 13(2), 134–143. DOI: 10.1038/sj.jea.7500262.
- de Prado Bert, P., Mercader, E. M. H., Pujol, J., Sunyer, J., & Mortamais, M. (2018). The Effects of Air Pollution on the Brain: a Review of Studies Interfacing Environmental Epidemiology and Neuroimaging. In Current environmental health reports (Vol. 5, Issue 3, pp. 351–364). NLM (Medline). DOI: 10.1007/s40572-018-0209-9.
- Eeftens, M., Beelen, R., de Hoogh, K., Bellander, T., Cesaroni, G., Cirach, M., Declercq, C., Dèdelè, A., Dons, E., de Nazelle, A., Dimakopoulou, K., Eriksen, K., Falq, G., Fischer, P., Galassi, C., Gražulevičienè, R., Heinrich, J., Hoffmann, B., Jerrett, M., Keidel, D., Korek, M., Lanki, T., Lindley, S., Madsen, C., Mölter, A., Nádor, G., Nieuwenhuijsen, M., Nonnemacher, M., Pedeli, X., Raaschou-Nielsen, O., Patelarou, E., Quass, U., Ranzi, A., Schindler, C., Stempfelet, M., Stephanou, E., Sugiri, D., Tsai, M.-Y., Yli-Tuomi, T., Varró, M.J., Vienneau, D., Klot, S.V., Wolf, K., Brunekreef, B., Hoek, G., 2012. Development of Land Use Regression Models for PM 2.5, PM 2.5 Absorbance, PM 10 and PM _{coarse} in 20 European Study Areas; Results of the ESCAPE Project. Environ. Sci. Technol. 46 (20), 11195–11205. https://doi.org/ 10.1021/es301948k.
- Folstein, M.F., Folstein, S.E., McHugh, P.R., 1975. "Mini-mental state": A practical method for grading the cognitive state of patients for the clinician. J. Psychiatr. Res. 12 (3), 189–198. https://doi.org/10.1016/0022-3956(75)90026-6.
- Frisoni, G. B., Boccardi, M., Barkhof, F., Blennow, K., Cappa, S., Chiotis, K., Démonet, J. F., Garibotto, V., Giannakopoulos, P., Gietl, A., Hansson, O., Herholz, K., Jack, C. R., Nobili, F., Nordberg, A., Snyder, H. M., Ten Kate, M., Varrone, A., Albanese, E., ... Winblad, B. (2017). Strategic roadmap for an early diagnosis of Alzheimer's disease based on biomarkers. In The Lancet Neurology (Vol. 16, Issue 8, pp. 661–676). Lancet Publishing Group. DOI: 10.1016/S1474-4422(17)30159-X.
- Genin, E., Hannequin, D., Wallon, D., Sleegers, K., Hiltunen, M., Combarros, O., Bullido, M.J., Engelborghs, S., De Deyn, P., Berr, C., Pasquier, F., Dubois, B., Tognoni, G., Fiévet, N., Brouwers, N., Bettens, K., Arosio, B., Coto, E., Del Zompo, M., Mateo, I., Epelbaum, J., Frank-Garcia, A., Helisalmi, S., Porcellini, E., Pilotto, A., Forti, P., Ferri, R., Scarpini, E., Siciliano, G., Solfrizzi, V., Sorbi, S., Spalletta, G., Valdivieso, F., Vepsäläinen, S., Alvarez, V., Bosco, P., Mancuso, M., Panza, F., Nacmias, B., Bossù, P., Hanon, O., Piccardi, P., Annoni, G., Seripa, D., Galimberti, D., Licastro, F., Soininen, H., Dartigues, J.-F., Kamboh, M.I., Van Broeckhoven, C., Lambert, J.C., Amouyel, P., Campion, D., 2011. APOE and Alzheimer disease: A major gene with semi-dominant inheritance. Mol. Psychiatry 16 (9), 903–907. https://doi.org/10.1038/mp.2011.52.
- Kanekiyo, T., Xu, H., Bu, G., 2014. ApoE and Aβ in Alzheimer's disease: accidental encounters or partners? Neuron 81 (4), 740–754. https://doi.org/10.1016/j. neuron.2014.01.045.
- Klunk, W.E., Koeppe, R.A., Price, J.C., Benzinger, T.L., Devous, M.D., Jagust, W.J., Johnson, K.A., Mathis, C.A., Minhas, D., Pontecorvo, M.J., Rowe, C.C., Skovronsky, D.M., Mintun, M.A., 2015. The Centiloid project: Standardizing quantitative amyloid plaque estimation by PET. Alzheimer's and Dementia 11 (1), 1–15.e4. https://doi.org/10.1016/j.jalz.2014.07.003.
- Liu, C., Kanekiyo, T., Xu, H., Bu, G., 2013. Apolipoprotein E and Alzheimer disease : risk, mechanisms and therapy. Nat. Rev. Neurol. 9, 106–118. https://doi.org/10.1038/ nrneurol.2012.263.
- Milà-Alomà, M., Salvadó, G., Gispert, J. D., Vilor-Tejedor, N., Grau-Rivera, O., Sala-Vila, A., Sánchez-Benavides, G., Arenaza-Urquijo, E. M., Crous-Bou, M., González-de-Echávarri, J. M., Minguillon, C., Fauria, K., Simon, M., Kollmorgen, G., Zetterberg, H., Blennow, K., Suárez-Calvet, M., & Molinuevo, J. L. (2020). Amyloid beta, tau, synaptic, neurodegeneration, and glial biomarkers in the preclinical stage of the Alzheimer's continuum. Alzheimer's & Dementia, in press, alz.12131. DOI: DOI: 10.1002/alz.12131.
- Milà-Alomà, M., Suárez-Calvet, M., Molinuevo, J.L., 2019. Latest advances in cerebrospinal fluid and blood biomarkers of Alzheimer's disease. In: Therapeutic Advances in Neurological Disorders, Vol. 12. SAGE Publications Ltd. https://doi. org/10.1177/1756286419888819
- Molinuevo, J.L., Gramunt, N., Gispert, J.D., Fauria, K., Esteller, M., Minguillon, C., Sánchez-Benavides, G., Huesa, G., Morán, S., Dal-Ré, R., Camí, J., 2016. The ALFA project: A research platform to identify early pathophysiological features of Alzheimer's disease. Alzheimer's & Dementia: Translational Research & Clinical Interventions 2 (2), 82–92. https://doi.org/10.1016/j.trci.2016.02.003.
- Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C., 2004. Translocation of inhaled ultrafine particles to the brain. Inhalation Toxicol. 16 (6–7), 437–445. https://doi.org/10.1080/08958370490439597.
- Oudin, A., Forsberg, B., Adolfsson, A.N., Lind, N., Modig, L., Nordin, M., Nordin, S., Adolfsson, R., Nilsson, L.-G., 2016. Traffic-Related Air Pollution and Dementia Incidence in Northern Sweden: A Longitudinal Study. Environ. Health Perspect. 124 (3), 306–312. https://doi.org/10.1289/ehp.1408322.
- Palmqvist, S., Mattsson, N., Hansson, O., & Alzheimer's Disease Neuroimaging Initiative. (2016). Cerebrospinal fluid analysis detects cerebral amyloid-β accumulation earlier than positron emission tomography. Brain : A Journal of Neurology, 139(Pt 4), 1226–1236. DOI: 10.1093/brain/aww015.
- Park, S.J., Lee, J., Lee, S., Lim, S., Noh, J., Cho, S.Y., Ha, J., Kim, H., Kim, C., Park, S., Lee, D.Y., Kim, E., 2020. Exposure of ultrafine particulate matter causes glutathione redox imbalance in the hippocampus: A neurometabolic susceptibility to Alzheimer's

S. Alemany et al.

pathology. Sci. Total Environ. 718, 137267. https://doi.org/10.1016/j. scitotenv.2020.137267.

- Peña-Casanova, J., Quiñones-Úbeda, S., Gramunt-Fombuena, N., Quintana-Aparicio, M., Aguilar, M., Badenes, D., Cerulla, N., Molinuevo, J. L., Ruiz, E., Robles, A., Barquero, M. S., Antúnez, C., Martínez-Parra, C., Frank-García, A., Fernández, M., Alfonso, V., Sol, J. M., & Blesa, R. (2009). Spanish multicenter normative studies (NEURONORMA project): Norms for verbal fluency tests. Archives of Clinical Neuropsychology, 24(4), 395–411. https://doi.org/10.1093/arclin/acp042.
- Peters, A., Veronesi, B., Calderón-Garcidueñas, L., Gehr, P., Chen, L. C., Geiser, M., Reed, W., Rothen-Rutishauser, B., Schürch, S., & Schulz, H. (2006). Translocation and potential neurological effects of fine and ultrafine particles a critical update. In Particle and Fibre Toxicology (Vol. 3). Part Fibre Toxicol. DOI: 10.1186/1743-8977-3-13.
- Quinones-Ubeda, S. (2009). Psychology. Ramon Llull University.
- Ramier, A.M., Hécaen, H., 1970. Respective rôles of frontal lesions and lesion lateralization in "verbal fluency" deficiencies. Revue Neurologique 123 (1), 17–22. https://pubmed.ncbi.nlm.nih.gov/5516326/.
- Reiman, E.M., Chen, K., Liu, X., Bandy, D., Yu, M., Lee, W., Ayutyanont, N., Keppler, J., Reeder, S.A., Langbaum, J.B., Alexander, G.E., Klunk, W.E., Mathis, C.A., Price, J.C., Aizenstein, H.J., DeKosky, S.T., Caselli, R.J., 2009. Fibrillar amyloid-beta burden in cognitively normal people at 3 levels of genetic risk for alzheimer's disease. Proc Natl Acad Sci U S A 106 (16), 6820–6825. https://doi.org/10.1073/ pnas.0900345106.
- Salvadó, G., Molinuevo, J.L., Brugulat-Serrat, A., Falcon, C., Grau-Rivera, O., Suárez-Calvet, M., Pavia, J., Niñerola-Baizán, A., Perissinotti, A., Lomeña, F., Minguillon, C., Fauria, K., Zetterberg, H., Blennow, K., Gispert, J.D., 2019. Centiloid cut-off values for optimal agreement between PET and CSF core AD biomarkers. Alzheimer's Res. Ther. 11 (1), 27. https://doi.org/10.1186/s13195-019-0478-z.
- Schikowski, T., Vossoughi, M., Vierkötter, A., Schulte, T., Teichert, T., Sugiri, D., Fehsel, K., Tzivian, L., Bae, I.S., Ranft, U., Hoffmann, B., Probst-Hensch, N., Herder, C., Krämer, U., Luckhaus, C., 2015. Association of air pollution with cognitive functions and its modification by APOE gene variants in elderly women. Environ. Res. 142, 10–16. https://doi.org/10.1016/j.envres.2015.06.009.

- Singh, P.P., Singh, M., Mastana, S.S., 2006. APOE distribution in world populations with new data from India and the UK. Ann. Hum. Biol. 33 (3), 279–308. https://doi.org/ 10.1080/03014460600594513.
- Snellman, A., Ekblad, L.L., Tuisku, J., Helin, S., Bucci, M., Karjalainen, T., Parkkola, R., Karrasch, M., Rinne, J.O., 2020. Effect of APOE-E4 gene dose on regional early neuroinflammation and beta-amyloid deposition in cognitively normal elderly volunteers. Alzheimer's & Dementia 16 (S4), e043359. https://doi.org/10.1002/ ALZ.043359.
- Sperling, R.A., Karlawish, J., Johnson, K.A., 2013. Preclinical Alzheimer disease The challenges ahead. Nat. Rev. Neurol. 9 (1), 54–58. https://doi.org/10.1038/ nrneurol.2012.241.
- Tzivian, L., Dlugaj, M., Winkler, A., Weinmayr, G., Hennig, F., Fuks, K.B., Vossoughi, M., Schikowski, T., Weimar, C., Erbel, R., Jöckel, K.-H., Moebus, S., Hoffmann, B., 2016. Long-Term Air Pollution and Traffic Noise Exposures and Mild Cognitive Impairment in Older Adults: A Cross-Sectional Analysis of the Heinz Nixdorf Recall Study. Environ. Health Perspect. 124 (9), 1361–1368. https://doi.org/10.1289/ ehp.1509824.
- Vert, C., Sánchez-Benavides, G., Martínez, D., Gotsens, X., Gramunt, N., Cirach, M., Molinuevo, J.L., Sunyer, J., Nieuwenhuijsen, M.J., Crous-Bou, M., Gascon, M., 2017. Effect of long-term exposure to air pollution on anxiety and depression in adults: A cross-sectional study. Int. J. Hyg. Environ. Health 220 (6), 1074–1080. https://doi. org/10.1016/j.ijheh.2017.06.009.
- Wang, R., Henderson, S.B., Sbihi, H., Allen, R.W., Brauer, M., 2013. Temporal stability of land use regression models for traffic-related air pollution. Atmos. Environ. 64, 312–319. https://doi.org/10.1016/j.atmosenv.2012.09.056.
- Younan, D., Petkus, A. J., Widaman, K. F., Wang, X., Casanova, R., Espeland, M. A., Gatz, M., Henderson, V. W., Manson, J. E., Rapp, S. R., Sachs, B. C., Serre, M. L., Gaussoin, S. A., Barnard, R., Saldana, S., Vizuete, W., Beavers, D. P., Salinas, J. A., Chui, H. C., ... Chen, J. C. (2020). Particulate matter and episodic memory decline mediated by early neuroanatomic biomarkers of Alzheimer's disease. Brain, 143(1), 289–302. DOI: 10.1093/brain/awz348.