Review

Ozone and Particulate Matter Exposure and Alzheimer's Disease: A Review of Human and Animal Studies

Rui-Ming Liu[a,*], Zechen Chong[b] and Jiu-Chiuan Chen[c]
[a]*Division of Pulmonary, Allergy, and Critical Care, Department of Medicine, the University of Alabama at Birmingham, Birmingham, AL, USA*
[b]*Department of Genetics, the University of Alabama at Birmingham, Birmingham, AL, USA*
[c]*Department of Biostatistics and Data Science, The University of Southern California, Los Angeles, CA, USA*

Abstract. Alzheimer's disease (AD), an aging-related neurodegenerative disease, is a major cause of dementia in the elderly. Although the early-onset (familial) AD is attributed to mutations in the genes coding for amyloid-β protein precursor (AβPP) and presenilin1/presenilin 2 (PS1/PS2), the cause for the late-onset AD (LOAD), which accounts for more than 95% of AD cases, remains unclear. Aging is the greatest risk factor for LOAD, whereas the apolipo protein E4 allele (*APOEε4*) is believed to be a major genetic risk factor in acquiring LOAD, with female *APOE ε4* carriers at highest risk. Nonetheless, not all the elderly, even older female *APOE ε4* carriers, develop LOAD, suggesting that other factors, including environmental exposure, must play a role. This review summarizes recent studies that show a potential role of environmental exposure, especially ozone and particulate matter exposure, in the development of AD. Interactions between environmental exposure, genetic risk factor (*APOE ε4*), and sex in AD pathophysiology are also discussed briefly. Identification of environmental risk factor(s) and elucidation of the complex interactions between genetic and environmental risk factors plus aging and female sex in the onset of AD will be a key to our understanding of the etiology and pathogenesis of AD and the development of the strategies for its prevention and treatment.

Keywords: Alzheimer's disease, animal models, epidemiology, ozone, particulate matters

INTRODUCTION

Alzheimer's disease (AD), a neurodegenerative disease, is a major cause of dementia in the elderly with healthcare costs tripling the general population age 65 or older. According to the Alzheimer's Association, 5.8 million Americans and 26.6 million people worldwide suffer from AD currently. With the increase in the lifespan, the prevalence of AD will be increased significantly. Despite extensive studies, there is still no effective treatment for this devastating disease due to an incomplete understanding of its etiology and pathogenesis. Early-onset (familial) AD, which accounts for less than 5% of AD cases, is attributed to mutations in the genes coding for amyloid-β protein precursor (AβPP) or presenilin1 (PS1) or presenilin2 (PS2), leading to increased production and deposition of amyloid-β peptide (Aβ) in the brain. The causes for the late-onset Alzheimer's disease (LOAD, also called sporadic AD), which

*Correspondence to: Rui-Ming Liu, Department of Medicine, the University of Alabama at Birmingham, Birmingham, AL 35294, USA. Tel.: +1 205 934 7028; Fax: +1 205 934 1721; E-mail: rliu@uab.edu.
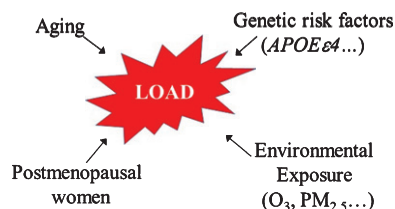
Fig. 1. Schematic flow chart of potential risk factors for the development of late-onset Alzheimer's disease (LOAD).

accounts for >95% of all AD cases, however, remain unclear.

Several risk factors for AD have been identified, including old age, carrying the apolipoprotein E4 (*APOE ε4*) allele, and being postmenopausal women (Fig. 1). Of 5.8 million Americans who have AD, 81% are age 75 or older [1]. It is estimated that the incidence of AD doubles every 5 years after age 65 and that 32% of the population at the age of 85 or older suffer from AD. Therefore, aging is considered to be the greatest risk factor for the development of AD [1–3]. Nonetheless, not all elderly suffer from AD, indicating that aging is a critical risk factor but alone is not sufficient to cause the disease. Human apolipoprotein E (apoE), existing in three isoforms (apoE2, apoE3, and apoE4), encoded by three distinct alleles *ε2*, *ε3*, and *ε4*, is a major carrier of lipids and cholesterol. Both epidemiology and animal studies suggest that the *APOE ε4* allele, which is carried by approximately 15% of the population worldwide [4–7], is a major genetic risk factor for AD [7, 8]. It has also been well documented that two-thirds of AD patients are women and postmenopausal women are at increased risk [9–11]. Importantly, *APOE ε4* has been shown to have more effect on the susceptibility of women to AD than it has on men [4, 11–16]. Nonetheless, not all of the *APOE ε4* carriers, even older female *APOE ε4* carriers, develop AD, suggesting that other factors, including environmental exposure, must play a role (Fig. 1).

Ozone (O_3) and particulate matters (PMs) are two of most abundant air pollutants in suburban and urban settings. Accumulated evidence from both epidemiology and animal studies suggests that exposure to unhealthy levels of O_3 and/or PMs, especially particulate matter with an aerodynamic diameter of 2.5 micrometers ($PM_{2.5}$) or smaller, may contribute to the onset of AD (Fig. 1). Nonetheless, which environmental pollutant(s) is (are) responsible and how environmental pollution contributes to the development of AD remains unclear. In this review, we summarized the recent findings that support a potential link between environmental exposure, especially O_3 and $PM_{2.5}$ exposure, and cognitive impairment. Interactions between environmental exposure, genetic risk factor *APOE ε4*, and sex in the development of AD are also discussed briefly.

AMBIENT OZONE EXPOSURE AND AD

O_3 is a highly reactive oxidant and one of the most abundant urban pollutants. Over 30% of the population in the United States live in areas with unhealthful levels of O_3 (American Lung Association State of the Air, 2012). Besides, some workers (e.g., pulp mills and outdoor construction) are intermittently exposed to relatively high levels of O_3 (0.3 ppm–1 ppm) through their working environment [17–24]. Although the lung is the primary target, emerging evidence from epidemiology and animal studies indicates that O_3 inhalation causes pathological changes in other tissues/organs beyond the respiratory system and that exposure to high levels of O_3 may be a risk factor for AD [25–37].

Evidence from human studies

Accumulation of $A\beta_{42}$ in the hippocampus and frontal cortex, the key brain regions required for learning and memory, is a pathological feature of AD. Several studies have shown that children and young adults who lived in the areas heavily polluted with O_3 and PMs, like Mexico City, had an increased level of $A\beta_{42}$ in their brains, compared with same-aged children and young adults who lived in low polluted areas [38–42]. Although the mechanism underlying the increased $A\beta$ load in the brain of the children/young adults living in heavily polluted areas is unclear, it has been reported that these children and young adults also have augmented neuroinflammation, disrupted blood-brain barriers, and damaged epithelial and endothelial cells in their brains compared to children and young adults who lived in clean air areas [38, 40–42]. These data suggest that dysfunction of endothelial and epithelial cells as well as blood-brain barriers may contribute to brain $A\beta$ accumulation in these children/young adults exposed to polluted air. It should be pointed out, although O_3 and PM are the major air pollutants in Mexico City, other types of pollutants also exist. None of these human studies have examined the correlation between neuropathological changes with the concentration of any

of these specific air pollutants. Therefore, the results from these studies can only suggest a potential link between air pollution and neuropathology in general and cannot distinguish the effects of O_3 and PM or other air pollutants.

To determine whether exposure to O_3 and/or PMs contributes to the onset of AD, several epidemiology studies have been conducted recently [25–30]. By analyzing the Neurobehavioural Evaluation System-2 (NES2) data from 1,764 adults participating in the Third National Health and Nutrition Examination Survey between 1988 and 1991 as well as the ambient PM10 (PM with aerodynamic diameter <10 μm) and O_3 data from EPA database, Chen et al. reported that there was no association between PM10 exposure and cognitive function measured by the NES-2 after adjustment for sociodemographic factors [25]. However, each 10 ppb increase in annual O_3 level was associated with 3.5–5.3 years of aging-related decline in cognitive performance [25]. Using a case-control comparison strategy, Wu et al. reported that long-term exposure to high concentrations of PM10 ($\geq 49.23\ \mu g/m^3$) or O_3 (≥ 21.56 ppb) was significantly associated with an increased risk of AD in Taiwan [26]. Gatto et al. examined cross-sectional associations between various ambient air pollutants, including O_3, $PM_{2.5}$, and nitrogen dioxide (NO_2), and six measures of cognitive function and global cognition among healthy, cognitively intact individuals residing in the Los Angeles basin. They found that exposure to high levels of $PM_{2.5}$ was associated with lower verbal learning, exposure to $NO_2 \geq 20$ ppb was associated with a lower logical memory, and exposure to 49 ppb of ambient O_3 was associated with a lower executive function [28]. By conducting a cohort study with 95,690 participants age ≥ 65, another group of scientists from Taiwan reported a weak association between ambient O_3 concentration and AD risk at baseline but a 211% increase in risk with every increase of 10.91 ppb of ambient O_3 concentration over the follow-up period from the year 2000 to the year 2010 [27]. Their studies suggest that long-term exposure to O_3 above the current US Environment Protection Agency (EPA) standards is associated with an increased risk of AD. In a huge population study aimed to evaluate the association between long-term exposure to air pollution and first hospitalization for dementia, Cerza et al. reported that there was a negative association between exposure to NO_2 and dementia hospitalization, whereas exposure to O_3 was positively associated with dementia hospitalization, especially senile dementia [30].

By assessing the cognitive performance of individuals from National Alzheimer's Coordinating Center, the average age of 76.8 years, and ambient O_3 and $PM_{2.5}$ concentrations established using a space-time Hierarchical Bayesian Model, Cleary et al. reported that the increased levels of O_3, but not $PM_{2.5}$, correlated with an increased rate of cognitive decline after adjustment for key individual and community-level risk factors [29]. Most interestingly, their data showed that individuals harboring one or more *APOE* ε4 alleles had a faster rate of cognitive decline and that the deleterious association of O_3 was confined to individuals with normal cognition when entering the study [29]. Their findings suggest that *APOE* ε4 may accelerate O_3 exposure-associated cognitive decline in the elderly and that healthy individuals are more sensitive to O_3 effect than memory-impaired individuals. Potential interaction between *APOE* ε4 and environmental exposure in the onset of AD will be further discussed later in this review. Together, these epidemiologic studies suggest that exposure to high levels of O_3 is a risk factor for AD in old population.

It should be pointed out that controversial results have also been reported [43, 44]. Using retrospective cohort from primary care data in London, Carey et al. reported that there was no association between exposure to higher levels of O3 and dementia, although they found that exposure to high concentrations of NO_2 or $PM_{2.5}$ was associated with dementia and that this association was more consistent for AD than vascular dementia [43]. In another large population study (approximate 2.1 million individuals) conducted in Ontario, Canada, Chen et al. reported that no association was found between O_3 exposure and the incidence of dementia between 2001 and 2013, although a positive association was found between exposure to PM2.5 and dementia incidence [44]. The reason for such a discrepancy between different population studies remains to be determined.

Evidence from animal studies

Epidemiology studies, although important, can only establish a correlation between an exposure and a disease, not a cause-effect relationship. Moreover, it is difficult to elucidate the underlying molecular mechanism from epidemiology studies. In contrast, animal studies can help establish a cause-effect relationship and dissect the molecular mechanisms underlying the pathophysiology. In this regard,

several studies have been conducted to explore the potential link between O_3 exposure and AD-like neuropathophysiology, using experimental animal models [31, 32, 34, 36, 45–49]. Rivas-Arancibia et al. showed that exposure to 0.2 ppm–1 ppm of O_3 for a short period (4 hours) impaired long-term memory, reduced dendritic spines, and induced brain lipid peroxidation in young rats [34, 45, 50]. This group also reported that O_3 exposure stimulated $A\beta_{42}$ production, impaired mitochondrial function, and reduced brain repair capacity, whereas treatment with antioxidant vitamin E attenuated O_3-induced hippocampal lipid peroxidation and memory deficits in rats [36, 46–48], suggesting that increased oxidative stress is responsible for O_3-induced memory deficit. Moreover, they have shown that O_3 exposure induced endoplasmic reticulum stress and apoptosis in rat hippocampus [49], suggesting that O_3 inhalation impairs memory through multiple mechanisms.

O_3 in the atmosphere (troposphere) is formed from chemical reactions between different air pollutants such as nitrogen oxides and volatile organic compounds (methane, organic solvents, etc.) under sunlight. Therefore, O_3 concentration in the atmosphere is high during the day and low in the evening. Moreover, several days of elevated O_3 are usually followed by a long period of "clean" air in most urban settings, although air pollution can last much longer time in heavily polluted areas such as Mexico City than in other areas. To mimic what occurs in the urban settings, we investigated, in a previous study, whether exposure to a cyclic O_3 exposure protocol, which consists of 5 days of O_3 exposure (0.8 ppm, 7 hours/day) followed by 9 days of filtered air exposure, may contribute to AD pathology, using AβPP/PS1 double transgenic mice, a well-established murine model of familial AD [31]. We found that exposure of 6-week-old non-transgenic littermates (wild type mice) to O_3 through a cyclic exposure protocol for 8 cycles had no significant effects on memory of either male and female wild type mice [31]. However, exposure of 6-week-old AβPP/PS1 mice to such a cyclic O_3 exposure protocol accelerated learning/memory function loss in male AβPP/PS1 mice, although it had no significant effect on female AβPP/PS1 mice [31]. We also found that male AβPP/PS1 mice had lower levels of antioxidants (glutathione and ascorbate) and augmented oxidative stress response (increased NADPH oxidase expression and lipid peroxidation) as well as increased neuronal apoptosis upon O_3 exposure, compared to female AβPP/PS1 mice [31]. On the other hand, female AβPP/PS1 mice had higher basal levels of $A\beta_{42}$ and $A\beta_{40}$ compared to male AβPP/PS1 mice; O_3 exposure had no significant effect on brain Aβ load in either sex. Together, our data suggest that exposure to O_3 under the conditions that mimic human exposure scenarios *per se* may not cause AD but can accelerate the pathophysiology of AD in genetically predisposed populations [31]. Our data also suggest that exposure to O_3 under the conditions mimic to human exposure scenarios impairs memory probably through inducing oxidative stress and neuronal cell death, rather than increasing brain Aβ accumulation.

Potential signals mediating lung-brain effects of O_3

The most challenging question that remains to be answered is how inhaled O_3 affects brain structure and function. In other words, what is (are) the signaling molecule(s) that mediate(s) the lung-brain effects of inhaled O_3? As a highly reactive oxidant, O_3 reacts quickly with multiple substrates in airway surface lining fluid and produces secondary reactive species such as ozonide radicals, singlet oxygen, antioxidant radical intermediates, and lipid peroxidation products [51, 52]. The extrapulmonary effects are most likely induced by these secondary reactive species rather than by O_3 itself. Several hypotheses have been proposed to explain the extrapulmonary effects of O_3, including inflammatory mediators [53], nitric oxide [54], and reactive lipid peroxidation product(s) [55] (Fig. 2). Erickson et al. reported that exposure of female BALB/c mice to 2 ppm O_3 for 2 hours increased the level of acute-phase serum amyloid A (A-SAA) protein in the liver, serum, and brain, although it had no significant effect on the blood levels of 23 cytokines/chemokines, except keratinocyte chemoattractant (KC/CXCL1), suggesting that A-SAA may be an important systemic signal of O_3 exposure to the CNS [56]. Mumaw et al. further showed that exposure to 1 ppm O_3 for 4 hours caused a persistent activation of brain microglia but had no significant effect on the levels of circulating cytokines such as CCL2, CCL11, TNFα, IL-6, and IL-1β in 8-weeks old Sprague-Dawley rats [57]. Interestingly, they showed that the serum from O_3 exposed mice induced an augmented immune response to LPS in hippocampal mixed glia cultures from old rats, compared to hippocampal glia cultures from young rats, and that monoclonal macrophage 1 antigen (MAC1) blocking antibodies eliminated O_3-induced priming effect [57]. These data suggest that O_3 exposure
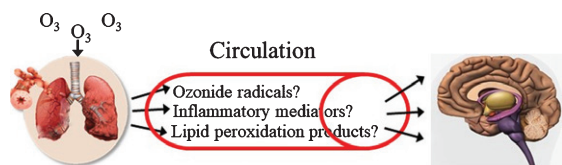
Fig. 2. Potential signal molecules involved in O_3 inhalation-induced brain pathophysiology.

leads to increases in non-cytokine-related signals in the circulation, which promote brain inflammatory responses [57].

Malondialdehyde (MDA) is a lipid peroxidation end product. Cretu et al. reported that exposure of rats to 0.5 ppm O_3 for 10 minutes daily for 2 or 4 weeks increased MDA level in the plasma and brain as well as pathological changes in the brain [58]. In a previous study, we showed that male AβPP/PS1 mice had lower levels of antioxidants (glutathione and ascorbate) and augmented induction of NADPH oxidase as well as increased neuronal apoptosis upon O_3 exposure, compared to female AβPP/PS1 mice [31]. This was associated with accelerated learning/memory impairment in male AβPP/PS1 mice [31]. We also found that O_3 inhalation increased the amounts of 4-hydroxynonenal (4HNE), an end product of lipid peroxidation, in the plasma and hippocampus/cortex of male, but not female, AβPP/PS1 mice [31]. Moreover, treatment of neuroblastoma SHSY5Y cells with 4HNE significantly increased apoptotic cell number, the activity of Caspase-3/7, and expression of p53 and Bax, three apoptosis markers [31]. Together, these data suggest that circulating lipid peroxidation products, such as MDA and 4HNE, which were released potentially from the oxidatively damaged lung, may contribute to brain pathological changes under O_3 inhalation condition [31, 58].

PARTICULATE MATTER EXPOSURE AND AD

Particulate matters (PMs) are liquid or solid matters suspended in the atmosphere. The major components of airborne particulates are sulfates, nitrates, ammonium, chloride, elementals, organic carbons, biological materials, and minerals, although the exact composition of PMs varies considerably depending on the location, weather, the season, time of day, emission sources, and many other factors [59]. Besides natural sources, such as volcanic activities and wildfires, human activities, including the combustion of fossil fuels, mining, and agricultural activities contribute importantly to PM pollution. PMs are arbitrarily grouped into ultrafine PMs with aerodynamic diameter <100 nm ($PM_{0.1}$), fine PMs with aerodynamic diameter 2.5 micrometers ($PM_{2.5}$), and coarse PMs with aerodynamic diameters between 2.5 and 10 microns (PM_{10}). As $PM_{2.5}$ and $PM_{0.1}$ can penetrate and deposit into the deep lung tissue and enter the circulation, these forms of PMs have greater potential to cause extrapulmonary diseases than PM_{10}. Accumulated evidence from human and animal studies suggests that exposure to particulate matters contributes importantly to cognitive decline and neuropathological changes [43, 44, 60–74].

Human studies

Emerging evidence from epidemiology studies suggests that exposure to PMs is an important environmental risk factor for the development of AD [26, 28, 43, 44, 62–67], although negative findings have also been reported [25, 29]. A longitudinal study conducted with 645 pairs of cognitively impaired subjects and their caregivers showed that aggravated neuropsychiatric symptoms were associated with exposure to high levels of $PM_{2.5}$ in South Korea [63]. A similar result was reported in another study conducted with 2,896 adult Korea aged 70 to 84 years [74]. The authors concluded that air pollutions, especially $PM_{2.5}$, were associated with cognitive impairment, including global cognition, attention, memory, and executive function in Korean old adults aged ≥70 years [74]. Through analysis of 130,978 adults aged from 50 to 79 years residing in London as well as the average annual concentrations of nitrogen dioxide (NO_2), $PM_{2.5}$, and O_3 at 20×20 m resolution from dispersion models, Carey et al. reported that there was a positive exposure-response relationship between dementia and NO_2 or $PM_{2.5}$, but not O_3, exposure and that the association was more consistent for AD than vascular dementia [43]. By following approximately 9.8 million subjects in 50 cities of the northeastern US, Kioumourtzouglou et al. reported that exposure to $PM_{2.5}$ is associated with significant increases in the hazard ratios for dementia, AD, and Parkinson's disease [61]. AD is associated with reduced hippocampal volume. Using brain-imaging data from UK Biobank, a large community-based dataset, and air pollution data, Hedges et al. showed that exposure to $PM_{2.5}$ was associated with a smaller left hippocampal volume, whereas none of the other

air pollutions, including $PM_{2.5-10}$, PM_{10}, nitrogen oxide, and nitrogen dioxide, was associated with hippocampal volume change [67]. By analyzing the data from four cohort studies conducted in Canada, Taiwan, the UK, and the US involving 12 million elderly aged ≥ 50 years, Tsai et al. found that exposure to a $10 \mu g/m^3$ increase in $PM_{2.5}$ from 2015 to 2018 was significantly and positively associated with increases in dementia and AD cases [64]. An early decline of episodic memory is reported in preclinical AD patients. To determine whether $PM_{2.5}$ exposure is associated with episodic memory decline and underlying neuroanatomic changes, Younan et al. conducted a longitudinal study using the data from Women's Health Initiative Study of Cognitive Aging and the Women's Health Initiative Memory Study of Magnetic Resonance Imaging [65]. Episodic memory was assessed by the California Vernal Learning Test, including measuring immediate free recall/new learning and delayed free recall, and by two brain MRI scans. A spatiotemporal model was used to estimate 3-year average $PM_{2.5}$ exposure before 1st MRI image analysis. Using multilevel structural equation models, they found that $PM_{2.5}$ was associated with greater declines in immediate recall and new learning but not in delayed-recall or composite scores [65]. Their findings suggest that $PM_{2.5}$ exposure is associated with memory decline at the preclinical stage [65].

Animal studies

Numerous studies in experimental animals have also shown that exposure to particulate matters induces neuroinflammation and AD-like pathology and impairs learning and memory [59, 71–73, 75–79]. Kim et al. reported that exposure of wild type mice to nickel nano particles increased brain levels of both $A\beta_{40}$ and $A\beta_{42}$ as well as the ratio of $A\beta_{42}$ to $A\beta_{40}$, a more disease-related change [71]. Bhatt et al. further showed that exposure of wild type mice (C57BL/6) to $PM_{2.5}$ for 9 months increased brain beta-site AβPP cleaving enzyme (BACE) protein level, AβPP processing, and $A\beta_{40}$ as well as cyclooxygenase 1 and 2 protein levels in mouse brain, although exposure to $PM_{2.5}$ for 2 months had no significant effects [75]. No significant effect of $PM_{2.5}$ exposure on the oxidative stress markers or synaptic marker was observed in the brain of these PM2.5 exposed wild type mice [75]. Some studies have been conducted to determine whether PM exposure accelerates neuropathological changes in AD model mice [73, 77].

Jew et al. reported that exposure of middle-aged (12.5 months old) mice to human exposure relevant concentrations of particulate matters (29–132 $\mu g/m^3$ of Harvard ultrafine concentrated ambient particles (HUCAPS) for 2 weeks significantly impaired spatial learning of both 3×TgAD mice, a murine model of familial AD, and wild type mice [73]. Jang et al. showed, on the other hand, that exposure Neuro2A cells in the *ex vivo* hippocampus tissue from 3×Tg-AD transgenic mice to fine PMs dose-dependently activated poly(ADP-ribose) polymerase (PARP-1), decreased NAD+, increased Aβ levels, and activates glial cells, mostly in CA1 region [77]. Inhibition of PAR-1 activity reversed PM-induced Aβ accumulation and glial activation, suggesting that increased PARP-1 activity is responsible for PM-induced AD pathological changes [77]. Human beings are usually exposed to multiple environmental agents simultaneously. Some of these agents exhibit synergistic, whereas others show additive or antagonist effects when exposed to them simultaneously. To test the potential synergistic effects between two air pollutants, Liu et al. exposed wild type C57BL/6 mice to formaldehyde (0.155 mg/kg/day) and $PM_{2.5}$ (0.193 mg/kg/day) alone or together for one week [72]. They found that exposure to either compound alone had little or no adverse effects on the mouse brain. However, when mice were exposed to $PM_{2.5}$ and formaldehyde simultaneously, AD-like pathological changes were observed, suggesting a synergistic effect between these two compounds [72]. Using GC-MS, LC-MS, western blotting, and immunohistochemistry techniques, Park et al. found that exposure to PMs induces alterations in metabolic pathways involved in redox homeostasis, neuroinflammation, and Aβ metabolism in the hippocampus, olfactory bulb, cortex, and cerebellum, with the changes in the hippocampus most significant [79]. Together, these studies suggest that PM exposure can impair memory through multiple mechanisms, including inducing oxidative stress, neuroinflammation, and brain Aβ accumulation.

TRAFFIC-RELATED EXPOSURE AND AD

Living near traffic roads is associated with exposure to many biohazardous chemicals as well as noise. Although exposure to particulate matters is one of the major concerns associated with traffic-related exposure, people living near roads are usually exposed to multiple hazardous chemicals besides particulate

matters, including nitrogen oxides, O_3, heavy metals, volatile organic compounds, and polycyclic aromatic hydrocarbons [80]. Accumulated evidence suggests that living near major traffic roads is associated with increased brain structure and function changes that may lead to neurodegenerative diseases such as AD and Parkinson's disease. Several large population studies and animal studies have been conducted to assess the effects of living near major traffic roads on the incidence of neurodegenerative diseases including AD [80–89]. In this section, we will specifically address the potential risk associated with traffic-related exposure in the development of AD as the effects from traffic-related exposure represent complex interactions between multiple air pollutants and noise and have been studied as an independent entity by many investigators.

Human population studies

Ranft et al. studied 399 women aged 68–79 years who lived for more than 20 years at the same residential address to address the relation between long-term exposure to traffic-related PMs and incidence of mild cognitive impairment [81]. After adjusted for potential confounders using regression analysis, they found distance (dose)-dependent effects of traffic-related particulate matters on neuropsychological performance [81]. Using a 15-year longitudinal human study data and annual mean nitrogen oxide data from the residential address of the participants as markers for traffic-related air pollution exposure, Oudin et al. demonstrated that there were dose-dependent increases in AD and vascular dementia in Northern Sweden [83]. By assessing two population-based cohorts with approximate 2.2 million people who resided in Ontario, Canada on April 1, 2001, Chen et al. further showed that the closer people lived to major traffic roads the higher the incidence of dementia, but not Parkinson's disease or multiple sclerosis, in Ontario, Canada [80]. These studies demonstrate a strong correlation between exposure to traffic-related pollutants and cognitive decline, although the exact disease-causing factors remain to be identified.

Animal studies

Aβ accumulation and tau protein phosphorylation in the hippocampus and frontal cortex are the pathological features of AD. Levesque et al. found that exposure of male Fisher 344 rats to diesel exhaust (DE) at concentrations of 992, 311, 100, 35, or 0 μg PM/m^3 for 6 months (subchronically) led to increased levels of inflammatory cytokine TNFα at high DE concentrations in all of the brain regions except the cerebellum [85]. No increase in IL-6 in any concentration of DE tested was observed, although IL-1β was increased in the groups treated with high concentrations of DE. They also showed that the levels of Aβ$_{42}$ and phosphorylated tau (pS^{199}) were significantly elevated at the higher concentrations of DE (992 and 311 μg PM/m^3) exposed groups in both temporal and frontal lobes, suggesting that DE exposure induces AD-like pathological changes [85]. Increased brain Aβ load has also been reported in mice after exposure to diesel engine exhaust or traffic-related air pollution [86, 87, 89]. Woodward et al. reported that exposure of young (3 months) and middle (18 months) aged mice to nanoscale PM (nPM) induced neurite atrophy, decreased myelin basic protein, and increased the amounts of Iba1, a microglial marker, and TNFα mRNA in the hippocampal CA1 region of young mice [87]. The dentate gyrus region, however, was unaffected [87]. Old control mice had similar changes as nPM exposed young mice and nPM exposure had no further effect on old mice, probably due to an age-ceiling effect [87]. In a recent study, the same group further showed that nPM exposure increased the levels of 4HNE, a lipid peroxidation product, and Aβ in lipid raft [89]. Treatment of mouse neuroblastoma N2a cells with nPM also caused dose-dependent increases in the levels of nitrogen oxide, 4HNE, and Aβ. Treatment with antioxidant N-acetyl-cysteine, on the other hand, attenuated nPM-induced oxidative stress responses and alterations of AβPP processing in lipid raft [89]. Their results suggest that traffic-related air pollutants promote neuronal pathological changes through inducing oxidative damage to lipid rafts [89].

INTERACTION BETWEEN GENE AND ENVIRONMENTAL EXPOSURE IN THE ONSET OF AD

It has been well documented that responses to environmental toxicants vary depending on the races, animal species, and even different individuals within the same race or species, suggesting that genetic background may affect the responses to environmental agents [90]. Besides aging, accumulating evidence indicates that genetic variations other than

mutations in AβPP and PS1 or PS2 genes, which lead to the development of early-onset (also called familial) AD, affect the susceptibility to the late-onset AD. As of 2018, 21 of AD-related susceptibility genes have been identified, which are involved in various biological processes, including lipid homeostasis, inflammation and immunity, endocytosis, apoptosis, and Aβ processing and clearance [90–96]. Of these identified susceptibility genes, the *APOE ε4* allele is believed to have the strongest link with AD [7]. Human apoE, existing in three isoforms (apoE2, apoE3, and apoE4), encoded by three distinct alleles *ε2*, *ε3*, and *ε4* is a major carrier of lipids and cholesterol. Both epidemiology and animal studies have shown that the *APOE ε4* allele, which is carried by approximately 15% of the population worldwide [4–7], is a major genetic risk factor for AD [4, 7, 8, 11–16]. Nonetheless, not all of the *APOE ε4* carriers, even in old ages, develop AD, suggesting that other risk factors, including environmental exposure, must play a role. It is believed that AD may develop as a consequence of complex interactions between aging, genetic risk factors, and environmental risk factors.In this section, we will further discuss such interactions in the development of AD, focusing on the *APOE ε4* allele.

Human studies

Mexico City is heavily polluted with $PM_{2.5}$ and O_3. Several studies have shown that Mexico City residents, including infants, children, and young adults, exhibit increased brain oxidative stress, inflammation, and AD-like pathological changes as well as cognitive deficits [70, 97–101]. Importantly, it has been reported that *APOE ε4* carriers have higher levels of these pathophysiological changes compared to non-*APOE ε4* carriers [97–101]. Calderon-Garciduenas et al. examined the brain metabolic rations, short-term memory, and IQ for 50 Mexico City residents with an average age of 13.4 years who carry either *APOE ε3* and *APOE ε4* allele [98]. They found that, compared to *APOE ε3* children, *APOE ε4* children had a decreased N-acetylaspartate/creatine ratios in the right frontal white matter and decrements on attention, short-term memory, and below-average scores in Verbal and Full-Scale IQ [98]. The same group of investigators also showed that infants, children, and young adults living in metropolitan Mexico City have increased cortical tau pretangles, neurofibrillary tangles, and amyloid in substantia nigra, auditory, oculomotor, trigeminal, and autonomic sys-

tems [101]. However, *APOE ε4* carriers have 23.6 times higher odds of neurofibrillary tangle stage V compared to non-*APOE ε4* carriers who have similar cumulative PM2.5 exposure and age [101]. This group of investigators also showed that *APOE ε4* heterozygous children (*APOE ε4/ε3*) have decreased attention and short-tern memory subscale and below-average scores in verbal, performance, and full-scale IQ compared to *APOE ε3/ε3* carriers [99]. Moreover, they showed that female heterozygous *APOE ε4* carriers with 75–94% of normal BMI are at the highest risk of severe cognitive deficits [99].

Several large population studies have also indicated that *APOE ε4* carriers are more sensitive than non-*APOE ε4* carriers to environmental exposure-induced cognitive decline [29, 102–104]. By assessing 5,419 participants with an average age of 76.8 years in the US, Cleary et al. reported that exposure to low-level of O_3 was associated with cognitive decline in the elderly and that individuals harboring one or more *APOE ε4* alleles exhibited a faster rate of cognitive decline compared to non-*APOE ε4* carriers [29]. They also found that the deleterious association of O_3 was confined to individuals with normal cognition who eventually became cognitively impaired [29]. Cacciottolo et al. reported that older women from the Women's Health Initiative Memory Study (WHIMS) who resided in places with fine PM exceeding EPA standards had increased risk of developing global cognitive decline and all-cause dementia with stronger adverse effects in *APOE ε4* carriers [102]. They further showed that exposure of female EFAD transgenic mice (5×FAD+/–/human *APOE ε3* or *ε4+/+*) to urban nanosized PM (nPM) for 15 weeks significantly increased cerebral Aβ load, which was worsened by *APOE ε4*.Their data suggest strongly that nPM exposure promotes AD pathological changes in the aging brain in women and that *APOE ε4* further enhances such sensitivity [102]. Alemany et al. reported that the associations between behavior/memory performance (behavior problem scores, caudate volume, and cognitive performance trajectories) and outdoor concentrations of polycyclic aromatic hydrocarbons and nitrogen dioxide were stronger in/limited to *APOE ε4* carriers [103]. A 6-year follow up study with 4,821 participants showed that high ambient concentrations of $PM_{2.5}$, NO_2, or PM_{10} were associated with a rapid decline of cognitive function and that the cognitive decline was faster in *APOE ε4* carriers than in non-*APOE ε4* carriers in responding to all of these pollutants [104]. Together, the evidence presented in these studies supports

a synergistic effect between *APOE ε*4 and environmental exposure in promoting memory decline or AD.

It should be mentioned that not all of the studies support such a synergistic effect. Oudin et al. reported that, although air pollution was associated with dementia incidence in a longitudinal study conducted in Northern Sweden, *APOE ε*4 has no significant effect on the outcome [105]. The discrepancies between these studies suggest that other factors, genetic and/or environmental, are involved as well. It should also be pointed out that none of these epidemiology studies has addressed sex-dependent interactions between *APOE* genotype and environmental exposure in AD pathophysiology. It is unclear whether *APOE ε*4 enhances the sensitivity to the environmental pollutants-induced memory impairment primarily in female *APOE ε*4 carriers or in both male and female *APOE ε*4 carriers. More studies are required to address this important issue.

Animal studies

Humanized apoE4 and apoE3 mice, also known as apoE3 or apoE4 targeted replacement (TR) mice, in which the endogenous murine *APOE* gene is replaced with the human *APOE ε*4 or *APOE ε*3 (common human form) gene, respectively [106], have been widely used to study the mechanisms by which *APOE ε*4 modulates AD pathophysiology. These mice have also been used to study the interactions between genetic risk factor *APOE ε*4 and dietary or environmental exposure in the development of AD [107–119], including particulate matters and O_3 exposure [32, 102]. Engstrom et al. reported that exposure to lead through drinking water for 12 weeks impaired memory in both apoE4 and apoE3 female mice but memory loss occurred earlier in apoE4 than in apoE3 mice [110]. ApoE4 mice, both male and female, are also more sensitive to cadmium-induced memory loss than apoE3 mice [119]. As in humans, the *APOE ε*4 allele mainly affects the memory of female mice under unchallenged conditions [8, 120–122]. Consequently, many studies have been conducted using female apoE4 TR mice only. Cacciottolo et al. reported that exposure of female EFAD (5XFAD$^{+/-}$/human *APOE ε*3 or ε4$^{+/+}$) transgenic mice to nanosized PM induced more dramatic AD-like pathological changes in the brain in apoE4 mice than it did in apoE3 mice [102].

Although some studies have shown that apoE ε4 TR mice are more sensitive to environmental factors-induced memory loss than apoE ε3 TR mice, opposite observations have also been reported [32, 109, 111]. Peris-Sampedro et al. found that exposure to an organophosphate pesticide chlorpyrifos impaired spatial memory in male apoE3, not male apoE4, TR mice [109]. Using different exposure strategy, the same group further showed that postnatal chlorpyrifos exposure impaired spatial memory of apoE3 but not apoE4 female mice [111]. In a recent study, we tested the hypothesis that O_3 exposure synergizes with the genetic risk factor *APOE ε*4 and aging leading to AD, using young and old male apoE4 and apoE3TR mice as men are more likely exposed to high levels of O_3 via working environments and few studies have addressed *APOE ε*4 effects on males [32]. We found, surprisingly, that O_3 exposure impaired memory only in old apoE3 male mice, while old apoE4 or young apoE3 and apoE4 male mice were spared [32]. Associated with memory loss, old apoE3 male mice exhibited increased protein oxidative modifications and neuroinflammation upon O_3 exposure compared to other groups [32]. In contrast to apoE3 male mice, old apoE4 male mice have significantly increased expression/activities of several enzymes involved in antioxidant defense, diminished protein oxidative modifications, and neuroinflammation upon O_3 exposure [32]. Together, these findings highlight the complexity of the interactions between *APOE* genotype, age, and environmental exposure in AD pathophysiology. These findings also suggest that the interactions between gene-environment in AD pathophysiology may be sex-dependent.

SEX, ENVIRONMENTAL EXPOSURE, *APOE ε*4, AND AD

Of the 5.6 million people age 65 or older who suffer from AD in the US, 3.5 million are women and 2.1 are men, indicating that the prevalence of AD is higher in women than in men [2, 14, 123–133]. The mechanism underlying increased susceptibility of old women to AD is unknown, although a loss of estrogen in menopause [126, 129, 130] and increased oxidative stress [134–140] is believed to contribute. Studies have shown that males and females respond differently to environmental pollutant-induced neurodevelopment and cognitive impairment [74, 141–146]. Moreover, it has been

reported that *APOE* ε4 differentially affects the susceptibility of males and females to environmental pollutant-induced memory decline [90, 110, 119, 143, 147]. It should be pointed out that controversial data have been reported. Some studies showed that *APOE* ε4 enhanced the sensitivity of females to environmental challenges, whereas other studies showed an opposite trend that *APOE* ε4 males were more sensitive than *APOE* ε4 females [110, 119]. Nonetheless, how sex affects the susceptibility to AD and how sex interacts with the *APOE* ε4 gene and environmental risk factors leading to AD remains largely unknown. In this section, we summarize a few published studies conducted in humans and animals, which address sex-related responses to environmental exposure in terms of neurodevelopment and cognitive function.

Although it has been long recognized that males and females respond differently to many challenges, very few studies have been conducted to dissect sex-dependent differences in response to environmental pollutant-induced neuropathophysiology. Prenatal exposure has been increasingly recognized to affect later-life susceptibility to some diseases. To explore the potential linkage between prenatal exposure to environmental pollutants and later life memory decline, Cowell et al. examined 258 mother-child dyads enrolled in a Boston, Massachusetts pregnancy cohort [142]. They found that, after stratification, the associations between prenatal exposure to traffic-related black carbon and memory scores were stronger in boys than in girls, although no significant effect was detected on learning and memory with non-stratified data [142]. Chiu et al. further showed that the responses to prenatal particulate exposure-induced neurodevelopment differ between boys and girls, depending on the time windows of prenatal exposure [146]. After adjusting for maternal age, education, race, and smoking status, they found that exposure to higher levels of $PM_{2.5}$ at 31–38 weeks was associated with lower IQ, at 20–26 weeks with increased omission errors, at 32–36 weeks with slower hit reaction time, and at 22–40 weeks with increased hit reaction time standard error among boys [146]. On the other hand, higher $PM_{2.5}$ exposure at 18–26 weeks was associated with reduced visual memory, and at 12–20 weeks with reduced general memory in girls [146]. Lertxundi et al. also found that prenatal exposure to $PM_{2.5}$ and NO_2 was negatively correlated with the neurological development of 4–6 years old children, especially boys [145]. In a recent study, we examined whether exposure to ambient fine particles (PM2.5) at residential locations affects intelligence quotient (IQ) during pre-/early- adolescence (ages 9–11) and emerging adulthood (ages 18–20) in a demographically-diverse population residing in Southern California [148]. We found that increased ambient PM2.5 levels were associated with decreased IQ scores, especially the Performance IQ (PIQ) and that the average PIQ score decreased by 3.08 points for each inter-quartile (7.73 µg/m3) increase in one-year PM2.5 preceding each assessment [148]. Interestingly, we found that the adverse effect was 89% stronger in males, compared to their counterparts [148]. Animal studies also showed that prenatal exposure to concentrated ambient ultrafine particles impaired the fixed interval schedule-controlled performance of male mice, while adult exposure impaired fixed interval of female mice, although concentrated ambient ultrafine particle exposure impaired short-term memory of both sexes [141]. Interestingly, it has been reported that the treatment of rats with estradiol prevents O_3-induced brain oxidative stress and social recognition impairment [35, 149], supporting the notion that a decrease in estrogen level may underling increased susceptibility of old females to O_3-induced AD-like pathophysiology.

Although it has been well documented that female *APOE* ε4 carriers have the highest risk of acquiring AD, how *APOE* genotype interacts with female sex leading to increased susceptibility to AD remains largely unknown. A few published studies also suggest that *APOE* ε4 may differentially affect the susceptibility of males and females to environmental pollutant-induced memory decline, although the underlying mechanism is unknown [90, 110, 119, 143, 147]. Some studies indicate that *APOE* ε4 enhances the sensitivity of females to environmental challenges [110], whereas other studies show an opposite trend [119]. Lead (Pb) is an important traffic-related environmental pollutant. It has been reported that Pb exposure impairs spatial working memory, assessed by novel object recognition test, in both male and female human *APOE* ε3 or *APOE* ε4 knock-in (ApoE ε4 and ApoE ε3 KI) mice [110]. ApoE ε4 KI mice, however, show memory impairment earlier than ApoE ε3 KI mice, with female ApoE ε4 KI mice manifesting memory deficit the earliest [110]. Cadmium (Cd) is another important environmental pollutant and a component of particulate matter. Studies have shown that exposure of ApoE ε4 KI and ApoE ε3 KI mice, both male and female, to Cd through drinking water impairs their spatial learning and memory function [119]. The memory deficits

manifested earlier in ApoE ε4 KI mice than in ApoE ε3 KI mice within the same sex [119]. Importantly, they showed that male ApoE ε3 and ApoE ε4 KI mice display memory deficits earlier than their corresponding female KI mice, suggesting that *APOE* ε4 affects the sensitivity of male mice to Cd-induced memory more than it does to female mice [119]. Nonetheless, more studies are warranted to dissect the complex interactions between *APOE* genotype, sex, and environmental exposure in the development of AD.

CONCLUSION

The etiology of LOAD is unknown. Accumulating evidence suggests that LOAD is caused most likely through complex interactions between genetic risk factors, environmental risk factors, and female sex plus aging. Both epidemiology and animal studies have shown that exposure to O_3 and PM is associated with cognitive decline; whether these air pollutants arethe culprit for AD, however, remains to be determined. Moreover, how female sex, *APOE* ε4, aging, and environmental risk factors interact leading to AD remains largely unknown and warrens further investigation. Understanding the complex interactions between these risk factors is a foundation for the development of strategies for the prevention and treatment of this devastating disease.

DISCLOSURE STATEMENT

Authors' disclosures available online (https://www.j-alz.com/manuscript-disclosures/20-0435r1).

REFERENCES

[1]   Hebert LE, Weuve J, Scherr PA, Evans DA (2013) Alzheimer disease in the United States (2010-2050) estimated using the 2010 census. *Neurology* **80**, 1778-1783.

[2]   Hebert LE, Bienias JL, Aggarwal NT, Wilson RS, Bennett DA, Shah RC, Evans DA (2010) Change in risk of Alzheimer disease over time. *Neurology* **75**, 786-791.

[3]   Kawas C, Gray S, Brookmeyer R, Fozard J, Zonderman A (2000) Age-specific incidence rates of Alzheimer's disease: The Baltimore Longitudinal Study of Aging. *Neurology* **54**, 2072-2077.

[4]   Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, Myers RH, Pericak-Vance MA, Risch N, van Duijn CM (1997) Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA* **278**, 1349-1356.

[5]   Singh PP, Singh M, Mastana SS (2006) APOE distribution in world populations with new data from India and the UK. *Ann Hum Biol* **33**, 279-308.

[6]   Eisenberg DT, Kuzawa CW, Hayes MG (2010) Worldwide allele frequencies of the human apolipoprotein E gene: Climate, local adaptations, and evolutionary history. *Am J Phys Anthropol* **143**, 100-111.

[7]   Liu CC, Liu CC, Kanekiyo T, Xu H, Bu G (2013) Apolipoprotein E and Alzheimer disease: Risk, mechanisms and therapy. *Nat Rev Neurol* **9**, 106-118.

[8]   Raber J, Wong D, Yu GQ, Buttini M, Mahley RW, Pitas RE, Mucke L (2000) Apolipoprotein E and cognitive performance. *Nature* **404**, 352-354.

[9]   Barron AM, Pike CJ (2012) Sex hormones, aging, and Alzheimer's disease. *Front Biosci (Elite Ed)* **4**, 976-997.

[10]   Association Association (2015) 2015 Alzheimer's disease facts and figures. *Alzheimers Dement* **11**, 332-384.

[11]   Riedel BC, Thompson PM, Brinton RD (2016) Age, APOE and sex: Triad of risk of Alzheimer's disease. *J Steroid Biochem Mol Biol* **160**, 134-147.

[12]   Damoiseaux JS, Seeley WW, Zhou J, Shirer WR, Coppola G, Karydas A, Rosen HJ, Miller BL, Kramer JH, Greicius MD, Alzheimer's Disease Neuroimaging Initiative (2012) Gender modulates the APOE epsilon4 effect in healthy older adults: Convergent evidence from functional brain connectivity and spinal fluid tau levels. *J Neurosci* **32**, 8254-8262.

[13]   Ungar L, Altmann A, Greicius MD (2014) Apolipoprotein E, gender, and Alzheimer's disease: An overlooked, but potent and promising interaction. *Brain Imaging Behav* **8**, 262-273.

[14]   Rocca WA, Mielke MM, Vemuri P, Miller VM (2014) Sex and gender differences in the causes of dementia: A narrative review. *Maturitas* **79**, 196-201.

[15]   Altmann A, Tian L, Henderson VW, Greicius MD, Alzheimer's Disease Neuroimaging Initiative (2014) Sex modifies the APOE-related risk of developing Alzheimer disease. *Ann Neurol* **75**, 563-573.

[16]   Sampedro F, Vilaplana E, de Leon MJ, Alcolea D, Pegueroles J, Montal V, Carmona-Iragui M, Sala I, Sanchez-Saudinos MB, Anton-Aguirre S, Morenas-Rodriguez E, Camacho V, Falcon C, Pavia J, Ros D, Clarimon J, Blesa R, Lleo A, Fortea J, Alzheimer's Disease Neuroimaging Initiative (2015) APOE-by-sex interactions on brain structure and metabolism in healthy elderly controls. *Oncotarget* **6**, 26663-26674.

[17]   Olin AC, Andersson I, Andersson M, Granung G, Hagberg S, Toren K (2004) Prevalence of asthma and exhaled nitric oxide are increased in bleachery workers exposed to ozone. *Eur Respir J* **23**, 87-92.

[18]   Mehta AJ, Henneberger PK, Toren K, Olin AC (2005) Airflow limitation and changes in pulmonary function among bleachery workers. *Eur Respir J* **26**, 133-139.

[19]   Henneberger PK, Olin AC, Andersson E, Hagberg S, Toren K (2005) The incidence of respiratory symptoms and diseases among pulp mill workers with peak exposures to ozone and other irritant gases. *Chest* **128**, 3028-3037.

[20]   Romieu I, Meneses F, Ramirez M, Ruiz S, Perez Padilla R, Sienra JJ, Gerber M, Grievink L, Dekker R, Walda I, Brunekreef B (1998) Antioxidant supplementation and respiratory functions among workers exposed to high levels of ozone. *Am J Respir Crit Care Med* **158**, 226-232.

[21]   O'Neill MS, Ramirez-Aguilar M, Meneses-Gonzalez F, Hernandez-Avila M, Geyh AS, Sienra-Monge JJ, Romieu

I (2003) Ozone exposure among Mexico City outdoor workers. *J Air Waste Manag Assoc* **53**, 339-346.

[22] Chan CC, Wu TH (2005) Effects of ambient ozone exposure on mail carriers' peak expiratory flow rates. *Environ Health Perspect* **113**, 735-738.

[23] Tuomi T, Engstrom B, Niemela R, Svinhufvud J, Reijula K (2000) Emission of ozone and organic volatiles from a selection of laser printers and photocopiers. *Appl Occup Environ Hyg* **15**, 629-634.

[24] Zhou JF, Chen WW, Tong GZ (2003) Ozone emitted during copying process–a potential cause of pathological oxidative stress and potential oxidative damage in the bodies of operators. *Biomed Environ Sci* **16**, 95-104.

[25] Chen JC, Schwartz J (2009) Neurobehavioral effects of ambient air pollution on cognitive performance in US adults. *Neurotoxicology* **30**, 231-239.

[26] Wu YC, Lin YC, Yu HL, Chen JH, Chen TF, Sun Y, Wen LL, Yip PK, Chu YM, Chen YC (2015) Association between air pollutants and dementia risk in the elderly. *Alzheimers Dement (Amst)* **1**, 220-228.

[27] Jung CR, Lin YT, Hwang BF (2015) Ozone, particulate matter, and newly diagnosed Alzheimer's disease: A population-based cohort study in Taiwan. *J Alzheimers Dis* **44**, 573-584.

[28] Gatto NM, Henderson VW, Hodis HN, St John JA, Lurmann F, Chen JC, Mack WJ (2014) Components of air pollution and cognitive function in middle-aged and older adults in Los Angeles. *Neurotoxicology* **40**, 1-7.

[29] Cleary EG, Cifuentes M, Grinstein G, Brugge D, Shea TB (2018) Association of low-level ozone with cognitive decline in older adults. *J Alzheimers Dis* **61**, 67-78.

[30] Cerza F, Renzi M, Gariazzo C, Davoli M, Michelozzi P, Forastiere F, Cesaroni G (2019) Long-term exposure to air pollution and hospitalization for dementia in the Rome longitudinal study. *Environ Health* **18**, 72.

[31] Akhter H, Ballinger C, Liu N, van Groen T, Postlethwait EM, Liu RM (2015) Cyclic ozone exposure induces gender-dependent neuropathology and memory decline in an animal model of Alzheimer's disease. *Toxicol Sci* **147**, 222-234.

[32] Jiang C, Stewart LT, Kuo HC, McGilberry W, Wall SB, Liang B, van Groen T, Bailey SM, Kim YI, Tipple TE, Jones DP, McMahon LL, Liu RM (2019) Cyclic O3 exposure synergizes with aging leading to memory impairment in male APOE epsilon3, but not APOE epsilon4, targeted replacement mice. *Neurobiol Aging* **81**, 9-21.

[33] Rivas-Arancibia S, Dorado-Martínez C, Borgonio-Pérez G, Hiriart-Urdanivia M, Verdugo-Diaz L, Durán-Vázquez A, Colin-Baranque L, Avila-Costa MR (2000) Effects of taurine on ozone-induced memory deficits and lipid peroxidation levels in brains of young, mature, and old rats. *Environ Res* **82**, 7-17.

[34] Dorado-Martinez C, Paredes-Carbajal C, Mascher D, Borgonio-Perez G, Rivas-Arancibia S (2001) Effects of different ozone doses on memory, motor activity and lipid peroxidation levels, in rats. *Int J Neurosci* **108**, 149-161.

[35] Guevara-Guzman R, Arriaga V, Kendrick KM, Bernal C, Vega X, Mercado-Gomez OF, Rivas-Arancibia S (2009) Estradiol prevents ozone-induced increases in brain lipid peroxidation and impaired social recognition memory in female rats. *Neuroscience* **159**, 940-950.

[36] Hernandez-Zimbron LF, Rivas-Arancibia S (2015) Oxidative stress caused by ozone exposure induces beta-amyloid 1-42 overproduction and mitochondrial accumulation by

activating the amyloidogenic pathway. *Neuroscience* **304**, 340-348.

[37] Bello-Medina PC, Prado-Alcala RA, Rivas-Arancibia S (2019) Effect of ozone exposure on dendritic spines of CA1 pyramidal neurons of the dorsal hippocampus and on object-place recognition memory in rats. *Neuroscience* **402**, 1-10.

[38] Calderon-Garciduenas L, Reed W, Maronpot RR, Henriquez-Roldan C, Delgado-Chavez R, Calderon-Garciduenas A, Dragustinovis I, Franco-Lira M, Aragon-Flores M, Solt AC, Altenburg M, Torres-Jardon R, Swenberg JA (2004) Brain inflammation and Alzheimer's-like pathology in individuals exposed to severe air pollution. *Toxicol Pathol* **32**, 650-658.

[39] Calderon-Garciduenas L, Franco-Lira M, Torres-Jardon R, Henriquez-Roldan C, Barragan-Mejia G, Valencia-Salazar G, Gonzalez-Maciel A, Reynoso-Robles R, Villarreal-Calderon R, Reed W (2007) Pediatric respiratory and systemic effects of chronic air pollution exposure: Nose, lung, heart, and brain pathology. *Toxicol Pathol* **35**, 154-162.

[40] Calderon-Garciduenas L, Solt AC, Henriquez-Roldan C, Torres-Jardon R, Nuse B, Herritt L, Villarreal-Calderon R, Osnaya N, Stone I, Garcia R, Brooks DM, Gonzalez-Maciel A, Reynoso-Robles R, Delgado-Chavez R, Reed W (2008) Long-term air pollution exposure is associated with neuroinflammation, an altered innate immune response, disruption of the blood-brain barrier, ultrafine particulate deposition, and accumulation of amyloid beta-42 and alpha-synuclein in children and young adults. *Toxicol Pathol* **36**, 289-310.

[41] Calderon-Garciduenas L, Kavanaugh M, Block M, D'Angiulli A, Delgado-Chavez R, Torres-Jardon R, Gonzalez-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 Alzheimers Dis* **28**, 93-107.

[42] Calderon-Garciduenas L, Reynoso-Robles R, Vargas-Martinez J, Gomez-Maqueo-Chew A, Perez-Guille B, Mukherjee PS, Torres-Jardon R, Perry G, Gonzalez-Maciel A (2016) Prefrontal white matter pathology in air pollution exposed Mexico City young urbanites and their potential impact on neurovascular unit dysfunction and the development of Alzheimer's disease. *Environ Res* **146**, 404-417.

[43] Carey IM, Anderson HR, Atkinson RW, Beevers SD, Cook DG, Strachan DP, Dajnak D, Gulliver J, Kelly FJ (2018) Are noise and air pollution related to the incidence of dementia? A cohort study in London, England. *BMJ Open* **8**, e022404.

[44] Chen H, Kwong JC, Copes R, Hystad P, van Donkelaar A, Tu K, Brook JR, Goldberg MS, Martin RV, Murray BJ, Wilton AS, Kopp A, Burnett RT (2017) Exposure to ambient air pollution and the incidence of dementia: A population-based cohort study. *Environ Int* **108**, 271-277.

[45] Avila-Costa MR, Colin-Barenque L, Fortoul TI, Machado-Salas P, Espinosa-Villanueva J, Rugerio-Vargas C, Rivas-Arancibia S (1999) Memory deterioration in an oxidative stress model and its correlation with cytological changes on rat hippocampus CA1. *Neurosci Lett* **270**, 107-109.

[46] Guerrero AL, Dorado-Martinez C, Rodriguez A, Pedroza-Rios K, Borgonio-Perez G, Rivas-Arancibia S (1999)

Effects of vitamin E on ozone-induced memory deficits and lipid peroxidation in rats. *Neuroreport* **10**, 1689-1692.

[47] Rivas-Arancibia S, Guevara-Guzman R, Lopez-Vidal Y, Rodriguez-Martinez E, Zanardo-Gomes M, Angoa-Perez M, Raisman-Vozari R (2010) Oxidative stress caused by ozone exposure induces loss of brain repair in the hippocampus of adult rats. *Toxicol Sci* **113**, 187-197.

[48] Rodriguez-Martinez E, Martinez F, Espinosa-Garcia MT, Maldonado P, Rivas-Arancibia S (2013) Mitochondrial dysfunction in the hippocampus of rats caused by chronic oxidative stress. *Neuroscience* **252**, 384-395.

[49] Rodriguez-Martinez E, Nava-Ruiz C, Escamilla-Chimal E, Borgonio-Perez G, Rivas-Arancibia S (2016) The effect of chronic ozone exposure on the activation of endoplasmic reticulum stress and apoptosis in rat hippocampus. *Front Aging Neurosci* **8**, 245.

[50] Rivas-Arancibia S, Vazquez-Sandoval R, Gonzalez-Kladiano D, Schneider-Rivas S, Lechuga-Guerrero A (1998) Effects of ozone exposure in rats on memory and levels of brain and pulmonary superoxide dismutase. *Environ Res* **76**, 33-39.

[51] Pryor WA, Squadrito GL, Friedman M (1995) The cascade mechanism to explain ozone toxicity: The role of lipid ozonation products. *Free Radic Biol Med* **19**, 935-941.

[52] Postlethwait EM, Cueto R, Velsor LW, Pryor WA (1998) O3-induced formation of bioactive lipids: Estimated surface concentrations and lining layer effects. *Am J Physiol* **274**, L1006-1016.

[53] Kafoury RM, Pryor WA, Squadrito GL, Salgo MG, Zou X, Friedman M (1999) Induction of inflammatory mediators in human airway epithelial cells by lipid ozonation products. *Am J Respir Crit Care Med* **160**, 1934-1942.

[54] Laskin DL, Pendino KJ, Punjabi CJ, Rodriguez del Valle M, Laskin JD (1994) Pulmonary and hepatic effects of inhaled ozone in rats. *Environ Health Perspect* **102 Suppl 10**, 61-64.

[55] Santiago-López D, Bautista-Martínez JA, Reyes-Hernandez CI, Aguilar-Martínez M, Rivas-Arancibia S (2010) Oxidative stress, progressive damage in the substantia nigra and plasma dopamine oxidation, in rats chronically exposed to ozone. *Toxicol Lett* **197**, 193-200.

[56] Erickson MA, Jude J, Zhao H, Rhea EM, Salameh TS, Jester W, Pu S, Harrowitz J, Nguyen N, Banks WA, Panettieri RA, Jr., Jordan-Sciutto KL (2017) Serum amyloid A: An ozone-induced circulating factor with potentially important functions in the lung-brain axis. *FASEB J* **31**, 3950-3965.

[57] Mumaw CL, Levesque S, McGraw C, Robertson S, Lucas S, Stafflinger JE, Campen MJ, Hall P, Norenberg JP, Anderson T, Lund AK, McDonald JD, Ottens AK, Block ML (2016) Microglial priming through the lung-brain axis: The role of air pollution-induced circulating factors. *FASEB J* **30**, 1880-1891.

[58] Cretu DI, Sovrea A, Ignat RM, Filip A, Bidian C, Cretu A (2010) Morpho-pathological and physiological changes of the brain and liver after ozone exposure. *Rom J Morphol Embryol* **51**, 701-706.

[59] Kilian J, Kitazawa M (2018) The emerging risk of exposure to air pollution on cognitive decline and Alzheimer's disease - Evidence from epidemiological and animal studies. *Biomed J* **41**, 141-162.

[60] Calderon-Garciduenas L, Avila-Ramirez J, Calderon-Garciduenas A, Gonzalez-Heredia T, Acuna-Ayala H, Chao CK, Thompson C, Ruiz-Ramos R, Cortes-Gonzalez V, Martinez-Martinez L, Garcia-Perez MA, Reis J, Mukherjee PS, Torres-Jardon 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 Alzheimers Dis* **54**, 597-613.

[61] Kioumourtzoglou MA, Schwartz JD, Weisskopf MG, Melly SJ, Wang Y, Dominici F, Zanobetti A (2016) Long-term PM2.5 exposure and neurological hospital admissions in the northeastern United States. *Environ Health Perspect* **124**, 23-29.

[62] Fu P, Guo X, Cheung FMH, Yung KKL (2019) The association between PM2.5 exposure and neurological disorders: A systematic review and meta-analysis. *Sci Total Environ* **655**, 1240-1248.

[63] Lee H, Kang JM, Myung W, Choi J, Lee C, Na DL, Kim SY, Lee JH, Han SH, Choi SH, Kim SY, Cho SJ, Yeon BK, Kim DK, Lewis M, Lee EM, Kim CT, Kim H (2019) Exposure to ambient fine particles and neuropsychiatric symptoms in cognitive disorder: A repeated measure analysis from the CREDOS (Clinical Research Center for Dementia of South Korea) study. *Sci Total Environ* **668**, 411-418.

[64] Tsai TL, Lin YT, Hwang BF, Nakayama SF, Tsai CH, Sun XL, Ma C, Jung CR (2019) Fine particulate matter is a potential determinant of Alzheimer's disease: A systemic review and meta-analysis. *Environ Res* **177**, 108638.

[65] Younan D, Petkus AJ, Widaman KF, Wang X, Casanova R, Espeland MA, Gatz M, Henderson VW, Manson JE, Rapp SR, Sachs BC, Serre ML, Gaussoin SA, Barnard R, Saldana S, Vizuete W, Beavers DP, Salinas JA, Chui HC, Resnick SM, Shumaker SA, Chen JC (2020) Particulate matter and episodic memory decline mediated by early neuroanatomic biomarkers of Alzheimer's disease. *Brain* **143**, 289-302.

[66] Li RL, Ho YC, Luo CW, Lee SS, Kuan YH (2019) Influence of PM2.5 exposure level on the association between Alzheimer's disease and allergic rhinitis: A national population-based cohort study. *Int J Environ Res Public Health* **16**, 3357.

[67] Hedges DW, Erickson LD, Kunzelman J, Brown BL, Gale SD (2019) Association between exposure to air pollution and hippocampal volume in adults in the UK Biobank. *Neurotoxicology* **74**, 108-120.

[68] Calderon-Garciduenas L, Mukherjee PS, Kulesza RJ, Torres-Jardon R, Hernandez-Luna J, Avila-Cervantes R, Macias-Escobedo E, Gonzalez-Gonzalez O, Gonzalez-Maciel A, Garcia-Hernandez K, Hernandez-Castillo A, Villarreal-Rios R, Research Universidad del Valle de Mexico UVMG (2019) Mild cognitive impairment and dementia involving multiple cognitive domains in Mexican urbanites. *J Alzheimers Dis* **68**, 1113-1123.

[69] Calderon-Garciduenas L, Herrera-Soto A, Jury N, Maher BA, Gonzalez-Maciel A, Reynoso-Robles R, Ruiz-Rudolph P, van Zundert B, Varela-Nallar L (2020) Reduced repressive epigenetic marks, increased DNA damage and Alzheimer's disease hallmarks in the brain of humans and mice exposed to particulate urban air pollution. *Environ Res* **183**, 109226.

[70] Calderon-Garciduenas L, Mukherjee PS, Waniek K, Holzer M, Chao CK, Thompson C, Ruiz-Ramos R, Calderon-Garciduenas A, Franco-Lira M, Reynoso-Robles R, Gonzalez-Maciel A, Lachmann I (2018) Non-phosphorylated tau in cerebrospinal fluid is a marker of Alzheimer's disease continuum in young urban-

ites exposed to air pollution. *J Alzheimers Dis* **66**, 1437-1451.

[71]   Kim SH, Knight EM, Saunders EL, Cuevas AK, Popovech M, Chen LC, Gandy S (2012) Rapid doubling of Alzheimer's amyloid-beta40 and 42 levels in brains of mice exposed to a nickel nanoparticle model of air pollution. *F1000Res* **1**, 70.

[72]   Liu X, Zhang Y, Luo C, Kang J, Li J, Wang K, Ma P, Yang X (2017) At seeming safe concentrations, synergistic effects of PM2.5 and formaldehyde co-exposure induces Alzheimer-like changes in mouse brain. *Oncotarget* **8**, 98567-98579.

[73]   Jew K, Herr D, Wong C, Kennell A, Morris-Schaffer K, Oberdorster G, O'Banion MK, Cory-Slechta DA, Elder A (2019) Selective memory and behavioral alterations after ambient ultrafine particulate matter exposure in aged 3xTgAD Alzheimer's disease mice. *Part Fibre Toxicol* **16**, 45.

[74]   Shin J, Han SH, Choi J (2019) Exposure to ambient air pollution and cognitive impairment in community-dwelling older adults: The Korean Frailty and Aging Cohort Study. *Int J Environ Res Public Health* **16**, 3767.

[75]   Bhatt DP, Puig KL, Gorr MW, Wold LE, Combs CK (2015) A pilot study to assess effects of long-term inhalation of airborne particulate matter on early Alzheimer-like changes in the mouse brain. *PLoS One* **10**, e0127102.

[76]   Dimakakou E, Johnston HJ, Streftaris G, Cherrie JW (2018) Exposure to environmental and occupational particulate air pollution as a potential contributor to neurodegeneration and diabetes: A systematic review of epidemiological research. *Int J Environ Res Public Health* **15**, 1704.

[77]   Jang S, Kim EW, Zhang Y, Lee J, Cho SY, Ha J, Kim H, Kim E (2018) Particulate matter increases beta-amyloid and activated glial cells in hippocampal tissues of transgenic Alzheimer's mouse: Involvement of PARP-1. *Biochem Biophys Res Commun* **500**, 333-338.

[78]   Shou Y, Huang Y, Zhu X, Liu C, Hu Y, Wang H (2019) A review of the possible associations between ambient PM2.5 exposures and the development of Alzheimer's disease. *Ecotoxicol Environ Saf* **174**, 344-352.

[79]   Park SJ, Lee J, Lee S, Lim S, Noh J, Cho SY, Ha J, Kim H, Kim C, Park S, Lee DY, Kim E (2020) Exposure of ultrafine particulate matter causes glutathione redox imbalance in the hippocampus: A neurometabolic susceptibility to Alzheimer's pathology. *Sci Total Environ* **718**, 137267.

[80]   Chen H, Kwong JC, Copes R, Tu K, Villeneuve PJ, van Donkelaar A, Hystad P, Martin RV, Murray BJ, Jessiman B, Wilton AS, Kopp A, Burnett RT (2017) Living near major roads and the incidence of dementia, Parkinson's disease, and multiple sclerosis: A population-based cohort study. *Lancet* **389**, 718-726.

[81]   Ranft U, Schikowski T, Sugiri D, Krutmann J, Kramer U (2009) Long-term exposure to traffic-related particulate matter impairs cognitive function in the elderly. *Environ Res* **109**, 1004-1011.

[82]   Gerlofs-Nijland ME, van Berlo D, Cassee FR, Schins RP, Wang K, Campbell A (2010) Effect of prolonged exposure to diesel engine exhaust on proinflammatory markers in different regions of the rat brain. *Part Fibre Toxicol* **7**, 12.

[83]   Oudin A, Forsberg B, Adolfsson AN, Lind N, Modig L, Nordin M, Nordin S, Adolfsson R, Nilsson LG (2016) Traffic-related air pollution and dementia incidence in northern Sweden: A longitudinal study. *Environ Health Perspect* **124**, 306-312.

[84]   Gonzalez-Maciel A, Reynoso-Robles R, Torres-Jardon R, Mukherjee PS, Calderon-Garciduenas L (2017) Combustion-derived nanoparticles in key brain target cells and organelles in young urbanites: Culprit hidden in plain sight in Alzheimer's disease development. *J Alzheimers Dis* **59**, 189-208.

[85]   Levesque S, Surace MJ, McDonald J, Block ML (2011) Air pollution & the brain: Subchronic diesel exhaust exposure causes neuroinflammation and elevates early markers of neurodegenerative disease. *J Neuroinflammation* **8**, 105.

[86]   Hullmann M, Albrecht C, van Berlo D, Gerlofs-Nijland ME, Wahle T, Boots AW, Krutmann J, Cassee FR, Bayer TA, Schins RPF (2017) Diesel engine exhaust accelerates plaque formation in a mouse model of Alzheimer's disease. *Part Fibre Toxicol* **14**, 35.

[87]   Woodward NC, Pakbin P, Saffari A, Shirmohammadi F, Haghani A, Sioutas C, Cacciottolo M, Morgan TE, Finch CE (2017) Traffic-related air pollution impact on mouse brain accelerates myelin and neuritic aging changes with specificity for CA1 neurons. *Neurobiol Aging* **53**, 48-58.

[88]   Espin-Perez A, Krauskopf J, Chadeau-Hyam M, van Veldhoven K, Chung F, Cullinan P, Piepers J, van Herwijnen M, Kubesch N, Carrasco-Turigas G, Nieuwenhuijsen M, Vineis P, Kleinjans JCS, de Kok T (2018) Short-term transcriptome and microRNAs responses to exposure to different air pollutants in two population studies. *Environ Pollut* **242**, 182-190.

[89]   Cacciottolo M, Morgan TE, Saffari AA, Shirmohammadi F, Forman HJ, Sioutas C, Finch CE (2020) Traffic-related air pollutants (TRAP-PM) promote neuronal amyloidogenesis through oxidative damage to lipid rafts. *Free Radic Biol Med* **147**, 242-251.

[90]   Eid A, Mhatre I, Richardson JR (2019) Gene-environment interactions in Alzheimer's disease: A potential path to precision medicine. *Pharmacol Ther* **199**, 173-187.

[91]   Hollingworth P, Harold D, Sims R, Gerrish A, Lambert JC, Carrasquillo MM, Abraham R, Hamshere ML, Pahwa JS, Moskvina V, Dowzell K, Jones N, Stretton A, Thomas C, Richards A, Ivanov D, Widdowson C, Chapman J, Lovestone S, Powell J, Proitsi P, Lupton MK, Brayne C, Rubinsztein DC, Gill M, Lawlor B, Lynch A, Brown KS, Passmore PA, Craig D, McGuinness B, Todd S, Holmes C, Mann D, Smith AD, Beaumont H, Warden D, Wilcock G, Love S, Kehoe PG, Hooper NM, Vardy ER, Hardy J, Mead S, Fox NC, Rossor M, Collinge J, Maier W, Jessen F, Ruther E, Schurmann B, Heun R, Kolsch H, van den Bussche H, Heuser I, Kornhuber J, Wiltfang J, Dichgans M, Frolich L, Hampel H, Gallacher J, Hull M, Rujescu D, Giegling I, Goate AM, Kauwe JS, Cruchaga C, Nowotny P, Morris JC, Mayo K, Sleegers K, Bettens K, Engelborghs S, De Deyn PP, Van Broeckhoven C, Livingston G, Bass NJ, Gurling H, McQuillin A, Gwilliam R, Deloukas P, Al-Chalabi A, Shaw CE, Tsolaki M, Singleton AB, Guerreiro R, Muhleisen TW, Nothen MM, Moebus S, Jockel KH, Klopp N, Wichmann HE, Pankratz VS, Sando SB, Aasly JO, Barcikowska M, Wszolek ZK, Dickson DW, Graff-Radford NR, Petersen RC, Alzheimer's Disease Neuroimaging I, van Duijn CM, Breteler MM, Ikram MA, DeStefano AL, Fitzpatrick AL, Lopez O, Launer LJ, Seshadri S, consortium C, Berr C, Campion D, Epelbaum J, Dartigues JF, Tzourio C, Alperovitch A, Lathrop M, consortium E, Feulner TM, Friedrich P, Riehle C, Krawczak M, Schreiber S, Mayhaus M, Nicolhaus S, Wagenpfeil S, Steinberg S, Stefansson H, Stefansson K,

Snaedal J, Bjornsson S, Jonsson PV, Chouraki V, Genier-Boley B, Hiltunen M, Soininen H, Combarros O, Zelenika D, Delepine M, Bullido MJ, Pasquier F, Mateo I, Frank-Garcia A, Porcellini E, Hanon O, Coto E, Alvarez V, Bosco P, Siciliano G, Mancuso M, Panza F, Solfrizzi V, Nacmias B, Sorbi S, Bossu P, Piccardi P, Arosio B, Annoni G, Seripa D, Pilotto A, Scarpini E, Galimberti D, Brice A, Hannequin D, Licastro F, Jones L, Holmans PA, Jonsson T, Riemenschneider M, Morgan K, Younkin SG, Owen MJ, O'Donovan M, Amouyel P, Williams J (2011) Common variants at ABCA7, MS4A6A/MS4A4E, EPHA1, CD33 and CD2AP are associated with Alzheimer's disease. *Nat Genet* **43**, 429-435.

[92] Naj AC, Jun G, Beecham GW, Wang LS, Vardarajan BN, Buros J, Gallins PJ, Buxbaum JD, Jarvik GP, Crane PK, Larson EB, Bird TD, Boeve BF, Graff-Radford NR, De Jager PL, Evans D, Schneider JA, Carrasquillo MM, Ertekin-Taner N, Younkin SG, Cruchaga C, Kauwe JS, Nowotny P, Kramer P, Hardy J, Huentelman MJ, Myers AJ, Barmada MM, Demirci FY, Baldwin CT, Green RC, Rogaeva E, St George-Hyslop P, Arnold SE, Barber R, Beach T, Bigio EH, Bowen JD, Boxer A, Burke JR, Cairns NJ, Carlson CS, Carney RM, Carroll SL, Chui HC, Clark DG, Corneveaux J, Cotman CW, Cummings JL, DeCarli C, DeKosky ST, Diaz-Arrastia R, Dick M, Dickson DW, Ellis WG, Faber KM, Fallon KB, Farlow MR, Ferris S, Frosch MP, Galasko DR, Ganguli M, Gearing M, Geschwind DH, Ghetti B, Gilbert JR, Gilman S, Giordani B, Glass JD, Growdon JH, Hamilton RL, Harrell LE, Head E, Honig LS, Hulette CM, Hyman BT, Jicha GA, Jin LW, Johnson N, Karlawish J, Karydas A, Kaye JA, Kim R, Koo EH, Kowall NW, Lah JJ, Levey AI, Lieberman AP, Lopez OL, Mack WJ, Marson DC, Martiniuk F, Mash DC, Masliah E, McCormick WC, McCurry SM, McDavid AN, McKee AC, Mesulam M, Miller BL, Miller CA, Miller JW, Parisi JE, Perl DP, Peskind E, Petersen RC, Poon WW, Quinn JF, Rajbhandary RA, Raskind M, Reisberg B, Ringman JM, Roberson ED, Rosenberg RN, Sano M, Schneider LS, Seeley W, Shelanski ML, Slifer MA, Smith CD, Sonnen JA, Spina S, Stern RA, Tanzi RE, Trojanowski JQ, Troncoso JC, Van Deerlin VM, Vinters HV, Vonsattel JP, Weintraub S, Welsh-Bohmer KA, Williamson J, Woltjer RL, Cantwell LB, Dombroski BA, Beekly D, Lunetta KL, Martin ER, Kamboh MI, Saykin AJ, Reiman EM, Bennett DA, Morris JC, Montine TJ, Goate AM, Blacker D, Tsuang DW, Hakonarson H, Kukull WA, Foroud TM, Haines JL, Mayeux R, Pericak-Vance MA, Farrer LA, Schellenberg GD (2011) Common variants at MS4A4/MS4A6E, CD2AP, CD33 and EPHA1 are associated with late-onset Alzheimer's disease. *Nat Genet* **43**, 436-441.

[93] Lambert JC, Grenier-Boley B, Harold D, Zelenika D, Chouraki V, Kamatani Y, Sleegers K, Ikram MA, Hiltunen M, Reitz C, Mateo I, Feulner T, Bullido M, Galimberti D, Concari L, Alvarez V, Sims R, Gerrish A, Chapman J, Deniz-Naranjo C, Solfrizzi V, Sorbi S, Arosio B, Spalletta G, Siciliano G, Epelbaum J, Hannequin D, Dartigues JF, Tzourio C, Berr C, Schrijvers EM, Rogers R, Tosto G, Pasquier F, Bettens K, Van Cauwenberghe C, Fratiglioni L, Graff C, Delepine M, Ferri R, Reynolds CA, Lannfelt L, Ingelsson M, Prince JA, Chillotti C, Pilotto A, Seripa D, Boland A, Mancuso M, Bossu P, Annoni G, Nacmias B, Bosco P, Panza F, Sanchez-Garcia F, Del Zompo M, Coto E, Owen M, O'Donovan M, Valdivieso F, Caffarra P, Scarpini E, Combarros O, Buee L, Campion D, Soini-

nen H, Breteler M, Riemenschneider M, Van Broeckhoven C, Alperovitch A, Lathrop M, Tregouet DA, Williams J, Amouyel P (2013) Genome-wide haplotype association study identifies the FRMD4A gene as a risk locus for Alzheimer's disease. *Mol Psychiatry* **18**, 461-470.

[94] Lambert JC, Ibrahim-Verbaas CA, Harold D, Naj AC, Sims R, Bellenguez C, DeStafano AL, Bis JC, Beecham GW, Grenier-Boley B, Russo G, Thorton-Wells TA, Jones N, Smith AV, Chouraki V, Thomas C, Ikram MA, Zelenika D, Vardarajan BN, Kamatani Y, Lin CF, Gerrish A, Schmidt H, Kunkle B, Dunstan ML, Ruiz A, Bihoreau MT, Choi SH, Reitz C, Pasquier F, Cruchaga C, Craig D, Amin N, Berr C, Lopez OL, De Jager PL, Deramecourt V, Johnston JA, Evans D, Lovestone S, Letenneur L, Morón FJ, Rubinsztein DC, Eiriksdottir G, Sleegers K, Goate AM, Fiévet N, Huentelman MW, Gill M, Brown K, Kamboh MI, Keller L, Barberger-Gateau P, McGuiness B, Larson EB, Green R, Myers AJ, Dufouil C, Todd S, Wallon D, Love S, Rogaeva E, Gallacher J, St George-Hyslop P, Clarimon J, Lleo A, Bayer A, Tsuang DW, Yu L, Tsolaki M, Bossù P, Spalletta G, Proitsi P, Collinge J, Sorbi S, Sanchez-Garcia F, Fox NC, Hardy J, Deniz Naranjo MC, Bosco P, Clarke R, Brayne C, Galimberti D, Mancuso M, Matthews F; European Alzheimer's Disease Initiative (EADI); Genetic and Environmental Risk in Alzheimer's Disease; Alzheimer's Disease Genetic Consortium; Cohorts for Heart and Aging Research in Genomic Epidemiology, Moebus S, Mecocci P, Del Zompo M, Maier W, Hampel H, Pilotto A, Bullido M, Panza F, Caffarra P, Nacmias B, Gilbert JR, Mayhaus M, Lannefelt L, Hakonarson H, Pichler S, Carrasquillo MM, Ingelsson M, Beekly D, Alvarez V, Zou F, Valladares O, Younkin SG, Coto E, Hamilton-Nelson KL, Gu W, Razquin C, Pastor P, Mateo I, Owen MJ, Faber KM, Jonsson PV, Combarros O, O'Donovan MC, Cantwell LB, Soininen H, Blacker D, Mead S, Mosley TH Jr, Bennett DA, Harris TB, Fratiglioni L, Holmes C, de Bruijn RF, Passmore P, Montine TJ, Bettens K, Rotter JI, Brice A, Morgan K, Foroud TM, Kukull WA, Hannequin D, Powell JF, Nalls MA, Ritchie K, Lunetta KL, Kauwe JS, Boerwinkle E, Riemenschneider M, Boada M, Hiltuenen M, Martin ER, Schmidt R, Rujescu D, Wang LS, Dartigues JF, Mayeux R, Tzourio C, Hofman A, Nöthen MM, Graff C, Psaty BM, Jones L, Haines JL, Holmans PA, Lathrop M, Pericak-Vance MA, Launer LJ, Farrer LA, van Duijn CM, Van Broeckhoven C, Moskvina V, Seshadri S, Williams J, Schellenberg GD, Amouyel P (2013) Meta-analysis of 74,046 individuals identifies 11 new susceptibility loci for Alzheimer's disease. *Nat Genet* **45**, 1452-1458.

[95] Dos Santos LR, Pimassoni LHS, Sena GGS, Camporez D, Belcavello L, Trancozo M, Morelato RL, Errera FIV, Bueno MRP, de Paula F (2017) Validating GWAS variants from microglial genes implicated in Alzheimer's disease. *J Mol Neurosci* **62**, 215-221.

[96] Lacour A, Espinosa A, Louwersheimer E, Heilmann S, Hernandez I, Wolfsgruber S, Fernandez V, Wagner H, Rosende-Roca M, Mauleon A, Moreno-Grau S, Vargas L, Pijnenburg YA, Koene T, Rodriguez-Gomez O, Ortega G, Ruiz S, Holstege H, Sotolongo-Grau O, Kornhuber J, Peters O, Frolich L, Hull M, Ruther E, Wiltfang J, Scherer M, Riedel-Heller S, Alegret M, Nothen MM, Scheltens P, Wagner M, Tarraga L, Jessen F, Boada M, Maier W, van der Flier WM, Becker T, Ramirez A, Ruiz A (2017) Genome-wide significant risk factors for Alzheimer's disease: Role in progression to dementia due to Alzheimer's

disease among subjects with mild cognitive impairment. *Mol Psychiatry* **22**, 153-160.

[97]   Calderon-Garciduenas L, de la Monte SM (2017) Apolipoprotein E4, gender, body mass index, inflammation, insulin resistance, and air pollution interactions: Recipe for Alzheimer's disease development in Mexico City young females. *J Alzheimers Dis* **58**, 613-630.

[98]   Calderon-Garciduenas L, Mora-Tiscareno A, Franco-Lira M, Zhu H, Lu Z, Solorio E, Torres-Jardon R, D'Angiulli A (2015) Decreases in short term memory, IQ, and altered brain metabolic ratios in urban Apolipoprotein epsilon4 children exposed to air pollution. *J Alzheimers Dis* **45**, 757-770.

[99]   Calderon-Garciduenas L, Jewells V, Galaz-Montoya C, van Zundert B, Perez-Calatayud A, Ascencio-Ferrel E, Valencia-Salazar G, Sandoval-Cano M, Carlos E, Solorio E, Acuna-Ayala H, Torres-Jardon R, D'Angiulli A (2016) Interactive and additive influences of Gender, BMI and Apolipoprotein 4 on cognition in children chronically exposed to high concentrations of PM2.5 and ozone. APOE 4 females are at highest risk in Mexico City. *Environ Res* **150**, 411-422.

[100] Calderon-Garciduenas L, Mora-Tiscareno A, Melo-Sanchez G, Rodriguez-Diaz J, Torres-Jardon R, Styner M, Mukherjee PS, Lin W, Jewells V (2015) A critical proton MR spectroscopy marker of Alzheimer's disease early neurodegenerative change: Low hippocampal NAA/Cr ratio impacts APOE varepsilon4 Mexico City children and their parents. *J Alzheimers Dis* **48**, 1065-1075.

[101] Calderon-Garciduenas L, Gonzalez-Maciel A, Reynoso-Robles R, Delgado-Chavez R, Mukherjee PS, Kulesza RJ, Torres-Jardon R, Avila-Ramirez J, Villarreal-Rios 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.

[102] Cacciottolo M, Wang X, Driscoll I, Woodward N, Saffari A, Reyes J, Serre ML, Vizuete W, Sioutas C, Morgan TE, Gatz M, Chui HC, Shumaker SA, Resnick SM, Espeland MA, Finch CE, Chen JC (2017) Particulate air pollutants, APOE alleles and their contributions to cognitive impairment in older women and to amyloidogenesis in experimental models. *Transl Psychiatry* **7**, e1022.

[103] Alemany S, Vilor-Tejedor N, Garcia-Esteban R, Bustamante M, Dadvand P, Esnaola M, Mortamais M, Forns J, van Drooge BL, Alvarez-Pedrerol M, Grimalt JO, Rivas I, Querol X, Pujol J, Sunyer J (2018) Traffic-related air pollution, APOEepsilon4 Status, and neurodevelopmental outcomes among school children enrolled in the BREATHE Project (Catalonia, Spain). *Environ Health Perspect* **126**, 087001.

[104] Kulick ER, Elkind MSV, Boehme AK, Joyce NR, Schupf N, Kaufman JD, Mayeux R, Manly JJ, Wellenius GA (2020) Long-term exposure to ambient air pollution, APOE-epsilon4 status, and cognitive decline in a cohort of older adults in northern Manhattan. *Environ Int* **136**, 105440.

[105] Oudin A, Andersson J, Sundstrom A, Nordin Adolfsson A, Oudin Astrom D, Adolfsson R, Forsberg B, Nordin M (2019) Traffic-related air pollution as a risk factor for dementia: No clear modifying effects of APOEvarepsilon4 in the Betula Cohort. *J Alzheimers Dis* **71**, 733-740.

[106] Knouff C, Hinsdale ME, Mezdour H, Altenburg MK, Watanabe M, Quarfordt SH, Sullivan PM, Maeda N (1999) Apo E structure determines VLDL clearance and atherosclerosis risk in mice. *J Clin Invest* **103**, 1579-1586.

[107] Arbones-Mainar JM, Johnson LA, Altenburg MK, Maeda N (2008) Differential modulation of diet-induced obesity and adipocyte functionality by human apolipoprotein E3 and E4 in mice. *Int J Obes (Lond)* **32**, 1595-1605.

[108] Huebbe P, Dose J, Schloesser A, Campbell G, Gluer CC, Gupta Y, Ibrahim S, Minihane AM, Baines JF, Nebel A, Rimbach G (2015) Apolipoprotein E (APOE) genotype regulates body weight and fatty acid utilization-Studies in gene-targeted replacement mice. *Mol Nutr Food Res* **59**, 334-343.

[109] Peris-Sampedro F, Basaure P, Reverte I, Cabre M, Domingo JL, Colomina MT (2015) Chronic exposure to chlorpyrifos triggered body weight increase and memory impairment depending on human apoE polymorphisms in a targeted replacement mouse model. *Physiol Behav* **144**, 37-45.

[110] Engstrom AK, Snyder JM, Maeda N, Xia Z (2017) Gene-environment interaction between lead and Apolipoprotein E4 causes cognitive behavior deficits in mice. *Mol Neurodegener* **12**, 14.

[111] Basaure P, Guardia-Escote L, Cabre M, Peris-Sampedro F, Sanchez-Santed F, Domingo JL, Colomina MT (2019) Learning, memory and the expression of cholinergic components in mice are modulated by the pesticide chlorpyrifos depending upon age at exposure and apolipoprotein E (APOE) genotype. *Arch Toxicol* **93**, 693-707.

[112] Nam KN, Wolfe CM, Fitz NF, Letronne F, Castranio EL, Mounier A, Schug J, Lefterov I, Koldamova R (2018) Integrated approach reveals diet, APOE genotype and sex affect immune response in APP mice. *Biochim Biophys Acta Mol Basis Dis* **1864**, 152-161.

[113] Slim KE, Vauzour D, Tejera N, Voshol PJ, Cassidy A, Minihane AM (2017) The effect of dietary fish oil on weight gain and insulin sensitivity is dependent on APOE genotype in humanized targeted replacement mice. *FASEB J* **31**, 989-997.

[114] Guardia-Escote L, Basaure P, Peris-Sampedro F, Biosca-Brull J, Cabre M, Sanchez-Santed F, Domingo JL, Colomina MT (2019) APOE genetic background and sex confer different vulnerabilities to postnatal chlorpyrifos exposure and modulate the response to cholinergic drugs. *Behav Brain Res* **376**, 112195.

[115] Basaure P, Guardia-Escote L, Biosca-Brull J, Blanco J, Cabre M, Peris-Sampedro F, Sanchez-Santed F, Domingo JL, Colomina MT (2019) Exposure to chlorpyrifos at different ages triggers APOE genotype-specific responses in social behavior, body weight and hypothalamic gene expression. *Environ Res* **178**, 108684.

[116] Tran TTT, Corsini S, Kellingray L, Hegarty C, Le Gall G, Narbad A, Muller M, Tejera N, O'Toole PW, Minihane AM, Vauzour D (2019) APOE genotype influences the gut microbiome structure and function in humans and mice: Relevance for Alzheimer's disease pathophysiology. *FASEB J* **33**, 8221-8231.

[117] Basaure P, Guardia-Escote L, Cabre M, Peris-Sampedro F, Sanchez-Santed F, Domingo JL, Colomina MT (2018) Postnatal chlorpyrifos exposure and apolipoprotein E (APOE) genotype differentially affect cholinergic expression and developmental parameters in transgenic mice. *Food Chem Toxicol* **118**, 42-52.

[118] Peris-Sampedro F, Reverte I, Basaure P, Cabre M, Domingo JL, Colomina MT (2016) Apolipoprotein E

(APOE) genotype and the pesticide chlorpyrifos modulate attention, motivation and impulsivity in female mice in the 5-choice serial reaction time task. *Food Chem Toxicol* **92**, 224-235.

[119] Zhang L, Wang H, Abel GM, Storm DR, Xia Z (2020) The effects of gene-environment interactions between cadmium exposure and Apolipoprotein E4 on memory in a mouse model of Alzheimer's disease. *Toxicol Sci* **173**, 189-201.

[120] Villasana L, Acevedo S, Poage C, Raber J (2006) Sex- and APOE isoform-dependent effects of radiation on cognitive function. *Radiat Res* **166**, 883-891.

[121] Bour A, Grootendorst J, Vogel E, Kelche C, Dodart JC, Bales K, Moreau PH, Sullivan PM, Mathis C (2008) Middle-aged human apoE4 targeted-replacement mice show retention deficits on a wide range of spatial memory tasks. *Behav Brain Res* **193**, 174-182.

[122] Rijpma A, Jansen D, Arnoldussen IA, Fang XT, Wiesmann M, Mutsaers MP, Dederen PJ, Janssen CI, Kiliaan AJ (2013) Sex differences in presynaptic density and neurogenesis in middle-aged ApoE4 and ApoE knockout mice. *J Neurodegener Dis* **2013**, 531326.

[123] Gao S, Hendrie HC, Hall KS, Hui S (1998) The relationships between age, sex, and the incidence of dementia and Alzheimer disease: A meta-analysis. *Arch Gen Psychiatry* **55**, 809-815.

[124] Andersen K, Launer LJ, Dewey ME, Letenneur L, Ott A, Copeland JR, Dartigues JF, Kragh-Sorensen P, Baldereschi M, Brayne C, Lobo A, Martinez-Lage JM, Stijnen T, Hofman A (1999) Gender differences in the incidence of AD and vascular dementia: The EURODEM Studies. EURODEM Incidence Research Group. *Neurology* **53**, 1992-1997.

[125] Di Carlo A, Baldereschi M, Amaducci L, Lepore V, Bracco L, Maggi S, Bonaiuto S, Perissinotto E, Scarlato G, Farchi G, Inzitari D (2002) Incidence of dementia, Alzheimer's disease, and vascular dementia in Italy. The ILSA Study. *J Am Geriatr Soc* **50**, 41-48.

[126] Vina J, Lloret A (2010) Why women have more Alzheimer's disease than men: Gender and mitochondrial toxicity of amyloid-beta peptide. *J Alzheimers Dis* **20Suppl 2**, S527-533.

[127] Li R, Singh M (2014) Sex differences in cognitive impairment and Alzheimer's disease. *Front Neuroendocrinol* **35**, 385-403.

[128] Mielke MM, Vemuri P, Rocca WA (2014) Clinical epidemiology of Alzheimer's disease: Assessing sex and gender differences. *Clin Epidemiol* **6**, 37-48.

[129] Scheyer O, Rahman A, Hristov H, Berkowitz C, Isaacson RS, Diaz Brinton R, Mosconi L (2018) Female sex and Alzheimer's risk: The menopause connection. *J Prev Alzheimers Dis* **5**, 225-230.

[130] Pike CJ (2017) Sex and the development of Alzheimer's disease. *J Neurosci Res* **95**, 671-680.

[131] Ferretti MT, Iulita MF, Cavedo E, Chiesa PA, Schumacher Dimech A, Santuccione Chadha A, Baracchi F, Girouard H, Misoch S, Giacobini E, Depypere H, Hampel H (2018) Sex differences in Alzheimer disease - the gateway to precision medicine. *Nat Rev Neurol* **14**, 457-469.

[132] Liu CC, Li CY, Sun Y, Hu SC (2019) Gender and age differences and the trend in the incidence and prevalence of dementia and Alzheimer's disease in Taiwan: A 7-year national population-based study. *Biomed Res Int* **2019**, 5378540.

[133] Ohta Y, Nomura E, Hatanaka N, Osakada Y, Matsumoto N, Sasaki R, Tsunoda K, Takemoto M, Tadokoro K, Hishikawa N, Wakutani Y, Yamashita T, Sato K, Omote Y, Abe K (2019) Female dominant association of sarcopenia and physical frailty in mild cognitive impairment and Alzheimer's disease. *J Clin Neurosci* **70**, 96-101.

[134] Prokai L, Prokai-Tatrai K, Perjesi P, Zharikova AD, Perez EJ, Liu R, Simpkins JW (2003) Quinol-based cyclic antioxidant mechanism in estrogen neuroprotection. *Proc Natl Acad Sci U S A* **100**, 11741-11746.

[135] Manthey D, Behl C (2006) From structural biochemistry to expression profiling: Neuroprotective activities of estrogen. *Neuroscience* **138**, 845-850.

[136] Ali SS, Xiong C, Lucero J, Behrens MM, Dugan LL, Quick KL (2006) Gender differences in free radical homeostasis during aging: Shorter-lived female C57BL6 mice have increased oxidative stress. *Aging Cell* **5**, 565-574.

[137] Aluise CD, Robinson RA, Beckett TL, Murphy MP, Cai J, Pierce WM, Markesbery WR, Butterfield DA (2010) Preclinical Alzheimer disease: Brain oxidative stress, Abeta peptide and proteomics. *Neurobiol Dis* **39**, 221-228.

[138] Santos RX, Correia SC, Zhu X, Smith MA, Moreira PI, Castellani RJ, Nunomura A, Perry G (2013) Mitochondrial DNA oxidative damage and repair in aging and Alzheimer's disease. *Antioxid Redox Signal* **18**, 2444-2457.

[139] Scheff SW, Ansari MA, Mufson EJ (2016) Oxidative stress and hippocampal synaptic protein levels in elderly cognitively intact individuals with Alzheimer's disease pathology. *Neurobiol Aging* **42**, 1-12.

[140] Berkowitz BA, Lenning J, Khetarpal N, Tran C, Wu JY, Berri AM, Dernay K, Haacke EM, Shafie-Khorassani F, Podolsky RH, Gant JC, Maimaiti S, Thibault O, Murphy GG, Bennett BM, Roberts R (2017) In vivo imaging of prodromal hippocampus CA1 subfield oxidative stress in models of Alzheimer disease and Angelman syndrome. *FASEB J* **31**, 4179-4186.

[141] Allen JL, Liu X, Weston D, Prince L, Oberdorster G, Finkelstein JN, Johnston CJ, Cory-Slechta DA (2014) Developmental exposure to concentrated ambient ultrafine particulate matter air pollution in mice results in persistent and sex-dependent behavioral neurotoxicity and glial activation. *Toxicol Sci* **140**, 160-178.

[142] Cowell WJ, Bellinger DC, Coull BA, Gennings C, Wright RO, Wright RJ (2015) Associations between prenatal exposure to black carbon and memory domains in urban children: Modification by sex and prenatal stress. *PLoS One* **10**, e0142492.

[143] Uchoa MF, Moser VA, Pike CJ (2016) Interactions between inflammation, sex steroids, and Alzheimer's disease risk factors. *Front Neuroendocrinol* **43**, 60-82.

[144] Jardim NS, Sartori G, Sari MHM, Muller SG, Nogueira CW (2017) Bisphenol A impairs the memory function and glutamatergic homeostasis in a sex-dependent manner in mice: Beneficial effects of diphenyl diselenide. *Toxicol Appl Pharmacol* **329**, 75-84.

[145] Lertxundi A, Andiarena A, Martinez MD, Ayerdi M, Murcia M, Estarlich M, Guxens M, Sunyer J, Julvez J, Ibarluzea J (2019) Prenatal exposure to PM2.5 and NO2 and sex-dependent infant cognitive and motor development. *Environ Res* **174**, 114-121.

[146] Chiu YH, Hsu HH, Coull BA, Bellinger DC, Kloog I, Schwartz J, Wright RO, Wright RJ (2016) Prenatal particulate air pollution and neurodevelopment in urban children:

Examining sensitive windows and sex-specific associations. *Environ Int* **87**, 56-65.

[147] Cacciottolo M, Christensen A, Moser A, Liu J, Pike CJ, Smith C, LaDu MJ, Sullivan PM, Morgan TE, Dolzhenko E, Charidimou A, Wahlund LO, Wiberg MK, Shams S, Chiang GC, Alzheimer's Disease Neuroimaging Initiative, Finch CE (2016) The APOE4 allele shows opposite sex bias in microbleeds and Alzheimer's disease of humans and mice. *Neurobiol Aging* **37**, 47-57.

[148] Wang P, Tuvblad C, Younan D, Franklin M, Lurmann F, Wu J, Baker LA, Chen JC (2017) Socioeconomic disparities and sexual dimorphism in neurotoxic effects of ambient fine particles on youth IQ: A longitudinal analysis. *PLoS One* **12**, e0188731.

[149] Angoa-Perez M, Jiang H, Rodriguez AI, Lemini C, Levine RA, Rivas-Arancibia S (2006) Estrogen counteracts ozone-induced oxidative stress and nigral neuronal death. *Neuroreport* **17**, 629-633.