

## DOCTORAL THESIS

### Causes of neurological disorders: associations of pm2.5 exposure and intestinal disorders

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## Abstract

**Objective** The aims of this project were to (a) perform a systematic review and meta-analysis of the associations between multiple neurological disorders (or neurological diseases) and potential influencing factors, including the association between fine particulate matter (PM<sub>2.5</sub>) and intestinal dysfunction, and (b) investigate the mechanisms and toxicological effects of PM<sub>2.5</sub> exposure in the brain and intestines using a mouse model of Alzheimer's disease (AD).

**Design** A systematic review and meta-analysis was conducted to assess the risks of PM<sub>2.5</sub> exposure, as manifested by the incidence of exposure-associated neurological disorders or intestinal dysfunction. An APP/PS1 transgenic mouse model for AD was used to study the brain damage resulting from PM<sub>2.5</sub> exposure, and the miRNA/mRNA regulatory mechanisms contributing to this damage. The inflammatory injuries and bacterial community changes in the intestines of AD mice exposed to PM<sub>2.5</sub> were also investigated.

**Data sources** Articles for systematic review and meta-analysis were obtained by searching PubMed and China National Knowledge Infrastructure (CNKI), which were published for more than ten years. Animal experiments were conducted at Shanxi University of Taiyuan in China, and toxicological tests were performed according to the stipulated methods and protocols.

**Review and experimental methods** Data on the risks of incidence of neurological disorders associated with the environmental factor (PM<sub>2.5</sub>) and biological factors (intestinal disorders and bacteria) were obtained, and random- or fixed-effects models (depending on the I<sup>2</sup> value) were used to pool the odds ratios (OR) with the 95% confidence intervals (CI) from individual studies. In the animal experiments, mice were divided into four groups of five animals per group, as follows: normal control mice in filtered air, AD mice in filtered air, normal control mice in PM<sub>2.5</sub> air, and AD mice in PM<sub>2.5</sub> air. PM<sub>2.5</sub> mice were exposed to ambient PM<sub>2.5</sub> in a whole-body inhalation exposure device for 8 weeks in Taiyuan, China. Well-established

methods were used to explore the toxicological mechanisms by which PM<sub>2.5</sub> exacerbated brain damage in AD mice, namely open-field testing, enzyme-linked immunosorbent assay (ELISA), real-time quantitative RT-PCR, hematoxylin-eosin (HE) staining, and transmission electron microscopy (TEM). Brain damage and related biomarkers in the brains were measured, and miRNA and mRNA profiles were detected using high-throughput sequencing methods. The signaling pathways of miRNAs or mRNAs were predicted and summarized, and specific miRNAs and mRNAs were screened to explore the possible regulatory mechanisms of PM<sub>2.5</sub>-induced brain damage in AD mice. Intestinal and fecal samples from these mice were also subjected to 16S rRNA gene sequencing. HE staining, ELISA, and metagenome bacterial diversity analyses were performed to investigate the effects of PM<sub>2.5</sub> inhalation on intestinal tissue damage, inflammatory responses, and changes of bacterial diversity and communities in AD mice.

**Results** Long-term PM<sub>2.5</sub> exposure has been associated with increased risks of stroke, dementia, AD, autism spectrum disorder (ASD), and Parkinson's disease (PD) in humans, with the risks of ischemic and hemorrhagic stroke being higher than that of stroke in general. Furthermore, a relatively higher risk of stroke has been observed in heavily polluted countries compared to less polluted countries. It is known that some intestinal disorders and related problems such as constipation, inflammatory bowel disease, irritable bowel syndrome, small intestinal bacterial overgrowth, and diarrhea significantly increase the risks of developing AD or PD. For example, the risk estimates of *Helicobacter pylori* infection were significantly associated with AD and PD. From another angle, preliminary animal experimental results showed that PM<sub>2.5</sub> promoted brain morphological damage and decreased spatial exploration ability in AD mice, and was concomitant with increases in the concentrations of amyloid- $\beta$ -42, acetylcholinesterase, tumor necrosis factor- $\alpha$ , and interleukin-6 and decreases in the concentrations of choline acetyltransferase. High-throughput sequencing and bioinformatics analyses revealed that miRNAs and mRNA had differential expression profiles subsequent to PM<sub>2.5</sub> exposure, which

suggested that these species are involved in the molecular regulatory mechanisms and possible signal pathways of PM<sub>2.5</sub>-aggravated brain injury in AD mice. These PM<sub>2.5</sub>-aggravated brain injuries were correlated with pathological intestinal injury, inflammatory responses, and changes in bacterial diversity in the intestines and feces of PM<sub>2.5</sub>-exposed AD mice, and decreases in predominant bacteria were identified. These data will assist in delineating the ability of PM<sub>2.5</sub> exposure to induce pathological changes in the brain and gut tissue via the brain-gut axis and thereby aggravate AD.

**Conclusions** A systematic review and meta-analysis showed that there is a significant association between PM<sub>2.5</sub> exposure and the occurrence of stroke, dementia, AD, ASD, and PD, and a strong association between intestinal disorders and the presence of certain bacteria and the development of AD and PD. PM<sub>2.5</sub> (environmental factors) and intestinal disorders accompanied by changes in bacterial diversity (internal biological factors) appeared to be the two most important factors that increase the risk of developing neurological disorders. Experimental animal data showed that PM<sub>2.5</sub> potently damaged the brain and intestines of AD mice, and that the toxicological mechanisms of this PM<sub>2.5</sub>-mediated brain injury led to morphological changes, inflammation, and perturbation of miRNA/mRNA regulation in the brain. These data suggest that PM<sub>2.5</sub> inhalation also have modulatory effects on the abundance and diversity of intestinal bacteria in AD mice. The findings of this study have clarified positive relationships between environmental and biological factors and neurological disorders and have elucidated the potential mechanisms by which PM<sub>2.5</sub> may mediate the initiation or exacerbation of AD.

**Keywords:** Neurological disorders, Alzheimer's disease, PM<sub>2.5</sub>, Intestinal disorders, Meta-analysis, Brain, Intestine, Toxicological effects

# Table of Contents

Declaration .....	i
Abstract .....	ii
Acknowledgements .....	v
Table of Contents .....	vi
List of Tables .....	x
List of Figures .....	xi
Chapter 1 Literature review of major neurological disorders and the potential factors affecting the development of these .....	1
1.1 Neurological disorders .....	1
1.1.1 Stroke .....	2
1.1.2 Dementia .....	3
1.1.3 Alzheimer's disease and mild cognitive impairment.....	3
1.1.4 Parkinson's disease.....	5
1.1.5 Autism spectrum disorder .....	7
1.2 Fine Particulate Matters Pollution.....	7
1.3 Objectives .....	8
Chapter 2 The association between PM <sub>2.5</sub> exposure and neurological disorders: a systematic review and meta-analysis .....	10
2.1 Abstract .....	10
2.2 Introduction.....	12
2.3 Methods.....	13
2.3.1 Search strategy and selection criteria.....	13
2.3.2 Data analysis.....	14
2.4 Results.....	16
2.5 Discussion .....	22
2.6 Conclusions.....	26
Chapter 3 Association of intestinal disorders with Parkinson's disease and Alzheimer's disease: A systematic review and meta-analysis.....	27
3.1 Abstract .....	27
3.2 Introduction.....	29
3.3 Methods.....	32
3.3.1 Search strategy and selection criteria.....	32

3.3.2 <i>Data analysis</i> .....	33
3.4 Results.....	34
3.5. Discussion.....	41
3.5.1 <i>Possible mechanisms underlying the induction of PD and AD associated with intestinal disorders</i> .....	41
3.5.2 <i>Possible mechanisms for the increased risks of PD and AD associated H. pylori infection</i> .....	46
3.6 Conclusions.....	49
Chapter 4 <i>PM<sub>2.5</sub> aggravates intestinal and brain injury and affects bacterial community structure of intestine and feces in Alzheimer’s disease transgenic mice</i> .....	50
4.1 Abstract.....	50
4.2 Introduction.....	52
4.3 Materials and Methods.....	54
4.3.2 <i>Animals and whole-body inhalation exposure to ambient PM<sub>2.5</sub></i> .....	55
4.3.3 <i>Histopathological analysis and ELISA</i> .....	56
4.3.4 <i>16S rRNA gene high-throughput sequencing and biological information analysis</i> .....	57
4.3.5 <i>Statistical analysis</i> .....	58
4.4 Results.....	58
4.4.1 <i>PM<sub>2.5</sub> concentrations and PM<sub>2.5</sub>-bound main component contents</i> .....	58
4.4.2 <i>Histopathology of intestine tissues in different groups</i> .....	58
4.4.3 <i>The TNF-<math>\alpha</math>, IL-6, and A<math>\beta</math>-42 levels in different groups</i> .....	62
4.4.4 <i>The abundance of OTUs of bacteria in the intestine and feces in different groups</i> .....	65
4.4.5 <i><math>\alpha</math>-diversity of the bacteria in the intestine and feces in different groups</i> .....	65
4.4.6 <i>Clustering analysis of bacterial communities</i> .....	66
4.4.7 <i>Distribution of dominant bacteria at different levels in the intestine and feces in four experimental groups</i> .....	71
4.4.8 <i>KEGG pathway analysis</i> .....	75
4.5 Discussion.....	77
4.5.1 <i>PM<sub>2.5</sub> caused pathological injury and inflammation in the brain and intestine in AD mice</i> .....	78
4.5.2 <i>Relationship between intestinal bacteria and inflammatory cytokines</i> .....	79
4.5.3 <i>PM<sub>2.5</sub> altered the bacterial community structure in the intestine and feces samples in AD mice</i> .....	80
4.5.4 <i>Functional prediction and analysis</i> .....	84
4.6 Conclusions.....	86

Chapter 5 An integrative analysis of miRNA and mRNA expression in the brains of Alzheimer’s disease transgenic mice exposed to PM <sub>2.5</sub> .....	87
5.1 Abstract .....	87
5.2 Introduction .....	89
5.3 Methods .....	91
5.3.1 <i>The collection and suspension preparation of pollutants</i> .....	91
5.3.2 <i>Mouse models and exposure system</i> .....	92
5.3.3 <i>Open field tests</i> .....	93
5.3.4 <i>Tissue extraction and subsequent analysis</i> .....	93
5.3.5 <i>TEM and immunohistochemistry analysis</i> .....	93
5.3.6 <i>ELISA</i> .....	94
5.3.7 <i>miRNA and mRNA expression profile analysis</i> .....	94
5.3.8 <i>Statistical analysis</i> .....	95
5.4 Results .....	96
5.4.1 <i>Ultrastructural changes to brain tissues in different groups</i> .....	97
5.4.2 <i>Expression changes of TNF-<math>\alpha</math>, IL-6, and A<math>\beta</math>-42</i> .....	98
5.4.3 <i>AChE and ChAT levels in the different experimental groups</i> .....	101
5.4.4 <i>Differential expression analysis of mRNA and miRNA in the brains of different group mice</i> .....	102
5.4.5 <i>Interaction of mRNA and miRNA expression profiles in different groups</i> .....	103
5.4.6 <i>GO and KEGG pathway analysis</i> .....	106
5.5 Discussion .....	108
5.5.1 <i>PM<sub>2.5</sub> induces brain-injury responses in AD mice</i> .....	108
5.5.2 <i>Integrative analysis of miRNA and mRNA expression profiling in PM<sub>2.5</sub>-AD mice</i> .....	110
5.5.2.1 <i>PM<sub>2.5</sub> changes miRNA expression patterns in PM<sub>2.5</sub>-AD mice’s brains</i> .....	110
5.5.2.2 <i>PM<sub>2.5</sub> affects the DE mRNA profile in PM<sub>2.5</sub>-AD mice’s brains</i> .....	110
5.5.2.3 <i>PM<sub>2.5</sub> influences miRNAs and their target genes in AD mice’s brains</i> .....	115
5.5.2.4 <i>GO pathways and KEGG pathways analysis in PM<sub>2.5</sub>-AD mice’s brain</i> .....	117
5.5.3 <i>Possible and potential causes of PM<sub>2.5</sub> composition in brain injuries of AD mice</i> .....	119
5.6 Conclusions .....	120
Chapter 6 Conclusions .....	121
List of References .....	125
Appendix .....	159
Appendix of Chapter 2 .....	159

Appendix of Chapter 3.....	171
Appendix of Chapter 4.....	211
Appendix of Chapter 5.....	230
Publications.....	243
Curriculum Vitae.....	248