Lead-exposure associated miRNAs in humans and Alzheimer’s disease: Potential biomarkers of the disease and disease processes

**Qingfeng Wen1,2, Marcha Verheijen1,2, Mandy Melissa Jane Wittens3,4,5, Julia Czuryło1, Sebastiaan Engelborghs3,4,5, Duncan Hauser1, Marcel HM van Herwijnen1, Thomas Lundh6, Ingvar A. Bergdahl7, Soterios A. Kyrtopoulos8, Theo M. De Kok1, Hubert J.M. Smeets1,2,9, Jacco Jan Briedé1,2, and Julian Krauskopf1**

1. Department of Toxicogenomics, Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, the Netherlands

2. MHeNS, School for Mental Health and Neuroscience, Maastricht University, Universiteitssingel 50, 6229 ER Maastricht, the Netherlands

3. Department of Biomedical Sciences Institute Born-Bunge, University of Antwerp, Universiteitsplein 1, BE-2610 Antwerpen, Belgium

4. Neuroprotection and Neuromodulation (NEUR), Center for Neurosciences (C4N), Vrije Universiteit Brussel (VUB), Laarbeeklaan 103, 1090 Brussel, Belgium

5. Department of Neurology, Universitair Ziekenhuis Brussel (UZ Brussel), Laarbeeklaan 101, 1090 Brussel, Belgium

6. Division of Occupational and Environmental Medicine, Lund University Hospital, Lund, Sweden

7. Section of Sustainable Health, Department of Public Health and Clinical Medicine, Umeå University, Umeå, Sweden

8. Institute of Chemical Biology, National Hellenic Research Foundation, Athens, Greece

9. School for Oncology and Developmental Biology (GROW), Maastricht University, Maastricht, the Netherlands

Correspondence and requests for materials should be addressed to Q.W. (email: q.wen@maastrichtuniversity.nl)

**Background**: Alzheimer’s disease (AD) is a neurodegenerative disease that eventually affects memory and behavior. The identification of biomarkers based on risk factors for AD helps understand the disease since the exact cause of AD remains unknown. Several studies have proposed microRNAs (miRNAs) in blood as potential biomarkers for AD. Exposure to heavy metals is a potential risk factor for onset and development of AD. Blood cells of subjects that are exposed to lead detected in the circulatory system, potentially reflect molecular responses to this exposure that are similar to the response of neurons.

**Keywords:** Alzheimer’s disease, lead exposure, miRNAs

**Methods:** First, we identified lead exposure related miRNAs based on 186 blood samples of general population from the EU’s Envirogenomarkers project (website), with known levels of heavy metals (lead, cadmium) in erythrocytes, using feature selection and linear mixed model. Second, we verified these miRNAs’ presence in human brain with a publicly available dataset (GSE157239), and identified their positively or negatively related gene targets and validated the expression of these targets in human brain with brain tissue samples (3 brain regions of 5 AD cases and 2 controls) from the EU Interreg Memories project (www.herinneringen.eu). Third, through pathway analysis, we analyzed relevant pathways that the targets of these miRNAs enriched in. Lastly, since some targets were transcription factors (TFs), to exhibit the complex regulation among miRNAs, gene targets and TFs, we developed the interacting regulation and visualized it via Cytoscape.

**Results**: A total of 4 miRNAs were identified as lead exposure-associated, with hsa-miR-3651, hsa-miR-150-5p and hsa-miR-664b-3p being negatively and hsa-miR-627 positively associated. All 4 miRNAs were detected in the human brain, of which 2 (miR-3651, miR-664b-3p) showed significant differential expression in AD brains versus controls, in accordance with the change direction of lead exposure. The miRNAs’ gene targets were found enriched in AD-relevant pathways such as axon guidance. Moreover, we identified several AD relevant transcription factors such as CREB1 associated with the identified miRNAs.These findings suggest that the identified miRNAs are involved in the development of AD and might be useful in the development of new, less invasive biomarkers for monitoring the risk on, and development of AD, including identification of novel targets for therapies.