Letter to the Editor

Association between Polymorphisms of *MALAT1* and Blood Lead Levels in Lead-exposed Workers*

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Lead, a heavy metal, which is nonessential but may be harmful to the human body, has been widely used to manufacture many products for use in the modern world. Lead-acid batteries have the advantages of low price, high safety, and advanced technology. Presently, our new energy policy is that electricity should replace fuels as an energy source. The use of lead-acid batteries continues to grow because of the development of electric vehicles and advancements in the automotive industry, meaning that lead-acid battery-producing companies still have a prominent position in China. However, the extensive use of lead usually leads to a series of health problems, such as damage to the hematopoietic system, nervous system, digestive system, urinary system, and reproductive system. Besides, lead emission by lead-acid battery factories into the air is considered a health hazard to residents of nearby communities[1]. In China, the Forum of National Occupational Disease reported that lead and its compounds were the major chemicals that were responsible for chronic occupational poisoning in 2015 and 2016. Thus, lead exposure is a very serious health hazard.

However, the toxic effects of lead and the clinical manifestations of lead-poisoning vary in different individuals. There are some differences in the biological markers and clinical symptoms among individuals who have been exposed to similar levels of lead. A previous study reported[2] that there was a significant association between the ALAD rs818708 polymorphism and the risk of lead poisoning. Tekin D et al.[3] reported that pregnant women with metallothionein 2A heterozygous AG genotype had higher BLLs than those with the AA homozygous genotype. These results indicated that, among lead-exposed workers, heterozygous genotype individuals are more susceptible to elevated BLLs than homozygous genotype individuals.

Long non-coding RNA (lncRNAs) are non-coding RNA (ncRNAs) with a length of more than 200 nucleotides. *MALAT1,* a lncRNA consisting of more than 8700 nt and located on chromosome 11q13, is localized to nuclear speckles, which contain a large number of nuclear proteins involved in mRNA splicing and transport. It has been shown that *MALAT1* plays an important role in cancer and acts as a transcriptional regulator for numerous genes, including those involved in cell proliferation, migration, and metastasis[4]. The relationship between *MALAT1* BLLs is unclear. Some studies have reported that lead could activate the MAPK signaling pathway to promote NGF-induced neurite outgrowth[5], indicating that lead can damage the nervous system through the MAPK signaling pathway. Moreover, Chen L found that the MAPK signaling pathway was inhibited in *MALAT1*-deficient N2a cells[6], suggesting a possible relationship between *MALAT1* and BLLs. Thus, our study intended to further explore the relationship between lncRNA *MALAT1* polymorphisms (rs3200401, rs619586, and rs11227209) and BLLs in lead-exposed workers.

Study Subjects All the 1,213 participants were lead-exposed workers mainly engaged in scrubber board, slice post, and tablet post. They were from five different battery factories, which were located in the Northern part of the Jiangsu Province, far from

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the cities and towns (at least 10 kilometers away), and no other factory has been sited within a 5-kilometer radius from the cities since January 2004. The employees were enrolled from the relatively nearby designated towns, with similar lifestyles and not living nearby the factories. These participants experienced a similar external lead exposure dose (CTWA = 0.025 ± 0.009 mg/m^3) during work. During the clinical evaluation, we excluded participants with a history of a hematological disorder, liver dysfunction, or kidney dysfunction, and participants who were on a lead-containing medical therapy were also excluded. All of them were informed about the details of this research and provided written informed consent before blood collection. Eighty-three participants were excluded from the study for not providing blood samples, or for unavoidable reasons. Eventually, 1,130 participants met our inclusion criteria.

Blood Lead Determination Approximately 2 mL of blood was sufficient for the measurement of the BLLs. BLLs were determined using graphite furnace atomic absorption spectrometry.

DNA Extraction The blood samples to be used for DNA extraction and genotyping were collected in tubes containing ethylenediaminetetraacetic acid (EDTA). Generally, 200 μL of blood was sufficient for the DNA extraction. DNA was extracted using a TIANamp Blood DNA kit (Tiangen, Nanjing, China). The enzyme-linked immunoassay was used to measure the DNA concentration. The extracted DNA and the remaining blood samples were stored at -80 °C for later use.

SNP Selection *MALAT1* SNPs were selected based on literature review and gene analysis using the Haploview 4.2 software. The following criteria were used to select the potential SNPs: 1) candidate SNPs should be located within the functional region of *MALAT1,* 2) minor allele frequency (MAF) for the selected SNPs should be greater than 0.05 in the Chinese Han Beijing (CHB) population, and 3) linkage disequilibrium value of r^2 should be less than 0.8 for candidate SNPs. Ultimately, three candidate SNPs of *MALAT1* met these criteria: rs11227209, rs3200401, and rs619586.

Polymorphism Genotyping Analysis Genotyping of the rs3200401, rs619586, and rs11227209 polymorphisms of *MALAT1* was performed using ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). Each reaction system for the genotyping contained 1.25 μL of distilled water, 2.625 μL PCR master mix (Roche, Branchburg, N.J., USA), 0.25 μL forward primer, 0.25 μL reverse primer, 0.125 μL FAM, 0.125 μL HEX (Applied Ji-ao Biosystems, Nanjing City, Jiangsu, China) and 0.5 μL of DNA. The reaction mixture was mixed and sequentially transferred onto 384 plates according to the manufacturer's instructions. The data for the genotyping were analyzed using the ABI 7900 system sequence detection software version 1.2.3 (Applied Biosystems).

Expression Quantitative Trait Loci We used the eQTL Browser of the University of Chicago for the expression quantitative trait loci (eQTLs) analysis.

Statistical Analysis $\bar{x}\pm s$ was used to describe the normal distribution of BLLs. We performed multiple linear regression to assess the correlation between *MALAT1* polymorphism genotypes and BLLs.

The general characteristics, including sex, age, marriage, education, smoking status, drinking status, and BLLs of the 1,130 participants are shown in Table 1.

Table 1. Demographic Characteristics and BLLs of Participants (*N* = 1,130)

Characteristics of Participants	*n* (%)
Sex	
Male	599 (53.0)
Female	531 (47.0)
Age (years)	
(20, 30)	83 (7.4)
(30, 40)	275 (24.3)
(40, 50)	619 (54.8)
(50, 60)	136 (12.0)
(60, 70)	17 (1.5)
Marriage	
Single	3 (0.2)
Married	1,113 (98.5)
Divorced	14 (1.3)
Education	
Illiterate	67 (5.9)
Literate, up to lower secondary level	158 (14.0)
High, up to middle secondary level	676 (59.8)
Higher, secondary level and above	229 (20.3)
Smoking status	
No	829 (73.4)
Yes	301 (26.6)
Drinking status	
No	817 (72.3)
Yes	313 (27.7)
BLL (μg/L)	
Mean ± SD	386.73 ± 177.93 (17-1,060)

***Note*.** BLL = blood lead level.