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Genetic variants in multisynthetase complex genes are associated with DNA damage levels in Chinese populations

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ABSTRACT

Aminoacyl-tRNA synthetases (ARSs) and ARS-interacting multi-functional proteins (AIMPs) form a multisynthetase complex (MSC) and play an important role in the process of DNA damage repair. We hypothesized that genetic variants in key *ARSs* and *AIMPs* might regulate the DNA damage response. Therefore, we systematically screened 23 potentially functional polymorphisms in MSC genes and evaluated the association between the genetic variants and DNA damage levels in 307 subjects from three cities in southern, central and northern China (Zhuhai, Wuhan and Tianjin, respectively). We examined personal 24-h $PM_{2.5}$ exposure levels and DNA damage levels in peripheral blood lymphocytes for each subject. We found that the variant allele of rs12199241 in *AIMP3* was significantly associated with DNA damage levels ($\beta = 0.343$, 95%CI: 0.133–0.554, $P = 0.001$). Meanwhile, the results of rs5030754 in *EPRS* and rs3784929 in *KARS* indicated their suggestive roles in DNA damage processes ($\beta = 0.331$, 95%CI: 0.062–0.599, $P = 0.016$ for rs5030754; $\beta = 0.192$, 95%CI: 0.016–0.368, $P = 0.033$ for rs3784929, respectively). After multiple testing, rs12199241 was still significantly associated with DNA damage levels. Combined analysis of these three polymorphisms showed a significant allele-dosage association between the number of risk alleles and higher DNA damage levels ($P_{trend} < 0.001$). These findings indicate that genetic variants in MSC genes may account for $PM_{2.5}$-modulated DNA damage levels in Chinese populations.

1. Introduction

Air pollution is a continuing challenge for public health worldwide, especially in China. Epidemiological studies have shown that particulate air pollution is associated with increased morbidity and mortality of various cardiopulmonary diseases, including lung cancer [1,2]. In addition, the International Agency for Research on Cancer (IARC) verified the carcinogenicity of air pollution in 2013 [3].

$PM_{2.5}$ is particulate matter ≤ 2.5 μm in aerodynamic diameter to which organic chemicals (such as polycyclic aromatic hydrocarbons, PAHs) and transition metals are adsorbed. Many studies have demonstrated that these components can generate oxygen free radicals and reactive oxygen species (ROS), which activate ROS-mediated DNA damage responses [4–7]. DNA damage may result in cell apoptosis, mutagenicity and genomic instability, and this damage plays a central role in the onset of carcinogenesis if unrepaired or repaired incorrectly [8–10].

ARSs (aminoacyl-tRNA synthetases) are fundamental enzymes that connect amino acids to their cognate tRNAs, thereby providing the building blocks for translation [11]. ARS-interacting multi-

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functional proteins (AIMPs, including AIMP1, AIMP2 and AIMP3) specifically bind to ARSs and are responsible for controlling the stability of ARSs and ensuring the fidelity and efficiency of translation [12]. ARSs and AIMPs are involved in a wide variety of functions, including transcription, translation, splicing, inflammation, angiogenesis, apoptosis, cell proliferation and DNA damage repair [13–15]. Notably, several ARSs and AIMPs are associated with DNA damage response by mediating apoptotic processes and nuclear translocation [16–19]. In mammals, eight different ARSs, including aspartyl-tRNA synthetase (DARS), bifunctional glutamyl-prolyl-tRNA synthetase (EPRS), isoleucyl-tRNA synthetase (IARS), lysyl-tRNA synthetase (KARS), leucyl-tRNA synthetase (LARS), methionyl-tRNA synthetase (MARS), glutaminyl-tRNA synthetase (QARS) and arginyl-tRNA synthetase (RARS), form a complex with the three AIMPs [12,20]. The complex is the multisynthetase complex (MSC). The MSC serves as a molecular reservoir that harbors component enzymes and cofactors until they are dispatched to the target sites when necessary [21].

In light of these evidences, we hypothesized that genetic variants in MSC genes might modify DNA damage levels for individuals exposed to $PM_{2.5}$. To determine the effects of variants on DNA damage, we systematically screened 23 potentially functional polymorphisms in MSC genes and performed a genetic association analysis in 307 Han Chinese subjects.

2. Materials and methods

2.1. Study subjects

This study was approved by the Ethics and Human Subject Committee of Tongji Medical College and Nanjing Medical University. In total, 328 subjects were recruited from three independent cohorts (119 from Zhuhai, 123 from Wuhan and 86 from Tianjin) in southern, central and northern China, respectively, with different $PM_{2.5}$ exposure levels. The basic information of enrolled subjects has been previously described [22]. Briefly, all subjects were disease-free genetically unrelated Han Chinese more than 40 years old, who had resided locally for more than 5 years. All participants were voluntary and signed informed consent before enrollment. Each subject was interviewed by trained interviewers using a structured questionnaire to collect demographic data and information on related factors including tobacco-based smoking and environment exposure history. After the interview, each participant donated approximately 5-mL peripheral venous blood for DNA extraction and examination of DNA damage. A total of 21 subjects were removed because they (i) declined to donate blood samples; (ii) failed to describe PM2.5 exposure information; or (iii) had poor DNA quality for chip analysis. In the end, 307 subjects from Zhuhai ($n = 110$), Wuhan ($n = 118$) and Tianjin ($n = 79$) were included in this study. The participants were all healthy on the day of examination. Individuals were defined as smokers if they had smoked an average of one cigarette or more per day for at least one year in their lifetime. Pack-years (py) of smoking were defined as packs per day multiplied by smoking duration years. The demographic and exposure information is shown in Supplementary Table 1. In brief, the mean age of subjects from Tianjin was higher than those from Zhuhai and Wuhan. $PM_{2.5}$ exposure levels varied across the three cities (median value: 68.35 $\mu g/m^3$, 114.96 $\mu g/m^3$ and 146.60 $\mu g/m^3$ for Zhuhai, Wuhan and Tianjin, respectively). Consistently with the $PM_{2.5}$ exposure levels, a similar pattern of DNA damage levels was observed with a median % Tail DNA of 1.36, 1.85 and 2.97 for subjects from Zhuhai, Wuhan and Tianjin, respectively.

2.2. $PM_{2.5}$ exposure level monitoring

Personal 24-h $PM_{2.5}$ exposure levels for each subject were measured with a $PM_{2.5}$ sampler and pump of Gilian5000 (Sensidyne Company, Florida, USA). The portable particle pump was placed in a small backpack and operated continuously during this period to collect $PM_{2.5}$. The $PM_{2.5}$ sampler was placed at the height of the respiratory zone of each subject. Sampling was performed on 37-mm Teflon filters (Beijing Lianyi Xingtong Apparatus & Instrument Co., Ltd., China) at a flow rate of 2.0 L/min. The filters were conditioned for 24 h before and after sampling, and this was followed by weighting. The personal $PM_{2.5}$ exposure level for each subject was represented by a 24-h average exposure concentration. The concentration was calculated based on the following equation, where C represents the mass concentrations of $PM_{2.5}$ ($\mu g/m3$), m_1 and m_2 is the mass of the Teflon filter before and after sampling, respectively (mg), V is the flow rate of sampling (L/min), and t is the duration of sampling (min):

$$C = \frac{m_2 - m_1}{V \times t}$$

2.3. Comet assay

The comet assay is widely used in environmental epidemiology studies to represent genetic toxicity effects of environmental exposure including $PM_{2.5}$ [6,23–25]. Within 4 h after blood sample collection, we isolated lymphocytes from 5.0-mL peripheral venous blood, which were again suspended in 5.0-mL ice-cold PBS (pH 7.4). The comet assay was performed according to Singh's method with minor modifications [26]. Three slides prepared for each subject, and for each slide, 50 lymphocytes were randomly selected to be analyzed. The cells were observed at 400 × magnification using a fluorescence microscope (Olympus, BX51). The percentage of tail DNA (% Tail) was regarded as the indicator of DNA damage levels and it was calculated by a computer-based image analysis system (version 1.0, IMI comet analysis software, China).

2.4. Functional polymorphisms selection and genotyping

Eight ARSs genes (*DARS*, *EPRS*, *IARS*, *KARS*, *LARS*, *MARS*, *QARS*, *RAR*,) and three ARS-interacting multi-functional proteins genes (*AIMP1*, *AIMP2*, *AIMP3*) that form the MSC were selected in our study. Based on the HapMap database (phase II + III Feb 09, on NCBI B36 assembly, dbSNP b126) and the HaploView 4.2 software, common SNPs (minor allele frequency (MAF) ≥ 5% in Chinese Han population) in these 11 genes were screened. After functional prediction from the SNPinfo Web Server (http://snpinfo.niehs.nih.gov/), a total of 42 potentially functional SNPs were selected. No functional SNPs were observed in *QARS*. Linkage disequilibrium (LD) analysis with an r^2 threshold of 0.8 was further applied to filter these functional SNPs. As a result, 26 functional SNPs were finally chosen for genotyping. Three SNPs were excluded because of design failure. Finally, 23 functional SNPs were included in this study.

Genomic DNA was isolated from leukocyte pellets of venous blood by proteinase K digestion followed by phenol–chloroform extraction and ethanol precipitation. All of the DNA samples were checked for quality and quantity by NanoDrop analysis and DNA electrophoresis before genotyping. The genotyping was performed with an Illumina Infinium® BeadChip (Illumina Inc.) and Genotype calling was performed using the GenTrain version 1.0 clustering algorithm in GenomeStudio V2011.1 (Illumina). All SNPs were successfully genotyped with call rates >98%.