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Original Article

Association of polymorphisms in the cytochrome P450 gene with susceptibility to lead poisoning in a chinese population

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Abstract: The present study is the first to explore the relationship between cytochrome P450 (CYP) polymorphisms and individual susceptibility to lead poisoning. Industrial workers (n = 1057) from five textile factories in east China provided venous blood for measurement of blood lead levels (BLL) and completed questionnaires. Genotyping was then performed for three selected SNPs (rs1048943, rs1056827, and rs1056836) from two CYP genes in blood samples from 287 lead poisoning cases (BLL > 400 μg/L) and 355 controls (BLL < 200 μg/L). Subsequently, the main effects of the genotype and its interactions were evaluated. Results revealed that Chinese individuals with the AA genotype of rs1048943 (OR = 3.03) and the haplotype ATC (rs1048943-rs1056827-rs1056836) (OR = 1.85) had an increased risk of lead poisoning. Multifactor dimensionality reduction analysis indicated that rs1048943-rs1056827 and rs1048943-rs1056827-rs1056836 had interactions and were related to increased lead poisoning risk (OR = 1.63 and 1.70, respectively). Overall, present findings indicate that *CYP1A1* genetic polymorphism, rs1048943, is associated with an increased risk of lead poisoning in a Chinese population. It may have potential as a biomarker for lead-exposed workers.

Keywords: Lead, *CYP1A1*, *CYP1B1*, single nucleotide polymorphism, haplotype

Introduction

Lead is a heavy metal widely used in industrial manufacturing, including production of batteries and chemicals. Lead is ubiquitous in the environment because it does not biodegrade. It may enter the human body via various routes, potentially causing harm to biological systems, including blood, nervous, and digestive systems. The cause of lead poisoning is clear but the presentation of toxicity varies between individuals. Under the same exposure conditions, some individuals will have signs and symptoms of lead poisoning, while others show only a slight elevation in blood lead concentrations. This suggests that genetic factors may contribute to susceptibility to lead poisoning. Supporting this, prior research has shown a relative relationship between ALAD gene polymorphisms and blood lead concentrations in lead poisoning [1, 2].

Cytochrome P450s (CYPs) constitute a major family of enzymes, predominantly located in the liver, which metabolize xenobiotics to non-toxic or carcinogenic metabolites. One of these enzymes, *CYP1A1*, is of major interest because of its role in bioactivating procarcinogens and environmental pollutants [3]. Korashy and El-Kadi provided evidence for the ability of lead to reduce *CYP1A1* induction by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in HepG2 cells through transcriptional and post-transcriptional mechanisms. They also demonstrated that heme oxygenase-1 (HO-1) is involved in the post-translational modification of *CYP1A1* expression by lead [4].

Increasing evidence has suggested that occupational exposure to open-cast coal mining residues, like dust particles, heavy metals, and polycyclic aromatic hydrocarbons (PAHs), may cause substantial DNA damage and genomic

Table 1. Demographic characteristics of study subjects

Variables	Cases (n = 287)		Controls (n = 355)		*P*
	n	%	n	%	
Sex					0.749[b]
Male	139	48.4	166	46.8	
Female	148	51.6	186	53.2	
Cigarette smoking					
Now	224	78.1	264	74.4	0.487[b]
Ever	13	4.5	22	6.2	
Never	50	17.4	69	19.4	
Alcohol consumption					0.873[b]
Now	230	80.1	283	79.7	
Ever	43	15.0	57	16.1	
Never	14	4.9	15	4.2	
Exposure time with lead (months)					
Mean ± SD	55.90 ± 2.82		56.37 ± 2.90		0.908[a]
Blood lead level (ug/L)					
Mean ± SD	513.57 ± 5.97		109.84 ± 3.08		< 0.001[a]

[a]*Student's* t-test. [b]Two-sided X^2 test.

instability that could contribute to cancer development and other work-related diseases. As a consequence of cellular metabolism, some of the intermediates and heavy metals found in blood samples from exposed individuals could be involved in the generation of oxidatively damaged DNA and proteins [5, 6]. Susceptibility to the hazardous actions of these chemicals may vary with genetic or acquired characteristics and may be associated with variations in genes that encode for carcinogen or xenobiotic-metabolizing enzymes, such as members of the CYP family [7]. Jover et al. demonstrated that the decrease in CYP enzyme function observed in lead-treated animals seemed to be a consequence of a mechanism involving reduced CYP transcription by lead and subsequent reduction in enzyme synthesis and activity [8].

However, associations between lead poisoning and CYP single nucleotide polymorphisms (SNPs) and their functional significance in the CYP gene have not yet been reported. Considering the vital functions of CYP in lead poisoning, it was speculated that polymorphisms in some of the CYP genes might influence genetic susceptibility to lead poisoning. The present report is a case-control study designed to elucidate the association between three *CYP1A1* and *CYP1B1* SNPs, rs1048943, rs1056827, and rs1056836, and genetic susceptibility to lead poisoning.

Subjects and methods

Subjects

This study was approved by the Institutional Review Board of Jiangsu Provincial Center for Disease Prevention and Control (Nanjing, Jiangsu Province, China). The study population consisted of 1,057 workers exposed to a similar external lead dose (0.017~0.004 mg/m^3) from five battery factories in Jiangsu Province, China. All workers started their lead-related employment after 2012 and had an orientation health check. Participants were excluded if they had any history of hematological disorders, liver or kidney dysfunction, or regularly had exposure to medicines containing lead. Each participant was interviewed by a trained staff member using a standardized questionnaire, including demographic information, detailed occupational and medical histories, and information on individual habits and symptoms. Participants with blood lead levels (BLL) > 400 μg/L were selected as the lead poisoning group (n = 287), while individuals with BLL < 200 μg/L were selected as the control group (n = 355).

Blood lead level measurement

Peripheral blood (3 mL) was collected into blood collection tubes, containing heparin sodium, for measurement of blood lead levels. Analysis of BLL occurred within 48 hours of collection. After addition of nitrate acid 0.2%, BLLs were measured by atomic absorption spectrometry using the PerkinElmer model 5000 graphite furnace atomic absorption spectrophotometer (PerkinElmer, Waltham, MA, USA). According to the Chinese standard, GBW09-139h-09140h and GBW (e) 09054b-09056b were used as controls for each BLL measurement. Each measurement was repeated by three individuals, independently, in a blinded

Table 2. General information of selected SNPs and Hardy-Weinberg test

Gene	SNP	Alleles	Chromosome	Functional Consequence	MAF		*P* for HWE[b]
					Subjects	Database[a]	
CYP1A1	rs1048943	A/G	15: 74720644	missense	0.253	0.232	0.114
CYP1B1	rs1056827	G/T	2: 38075034	missense	0.133	0.125	0.435
CYP1B1	rs1056836	C/G	2: 38071060	missense	0.123	0.128	0.610

[a]Data from NCBI dbSNP. [b]*P* value of Hardy-Weinberg test.

fashion. BLLs of samples with less than 5% concentration error were included.

DNA extractions

Peripheral blood (3 mL) was collected into blood collection tubes, containing ethylene diamine tetra acetic acid (EDTA), for DNA isolation and genotyping. DNA was extracted using the QIAcube HT and QIAamp 96 DNA QIAcube HT Kit (Qiagen, Dusseldorf, Germany), according to manufacturer protocol, and stored at -20°C until use.

SNP selection and genotyping

Target SNPs in *CYP1A1* and *CYP1B1* genes were selected based on the 1000 Genomes Project database and previous literature findings. Search criteria for SNPs were as follows: *a*) MAF (minor allele frequency) of CHB > 0.10; and *b*) Linkage disequilibrium value of $r^2 > 0.8$. In the end, twelve candidate SNPs were selected using these criteria by Haploview software. These candidate SNPs were then searched in PubMed, finding that rs1048943 (*CYP1A1*) and rs1056827 and rs1056836 (*CYP1B1*) were the most commonly reported SNPs of *CYP1A1* and *CYP1B1*. Ultimately, these three SNPs were selected for genotyping.

Genotypes for the selected polymorphisms were screened with ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA., USA) using pre-designed commercial genotyping methods. Extracted DNA and genotyping assays were added to the TaqMan universal PCR master mix (Roche, Branchburg, N.J., USA), according to the manufacturer instructions. Genotyping procedures were then performed by ABI 7900 real-time PCR system (Applied Biosystems, Foster City, CA., USA). Results were analyzed using ABI 7900 System sequence detection software version 1.2.3 (Applied Biosystems, Foster City, CA., USA).

Statistical analyses

Statistical analyses were performed using SPSS 24.0 software (Chicago, IL, USA). Goodness-of-fit χ^2 tests were conducted for the Hardy-Weinberg equilibrium rule of the SNPs in the CYP gene among control subjects. Categorical variables are presented as percentages and continuous variables are described as the mean ± standard error (SE). Odds ratios (ORs) and 95% confidence intervals (95% CI) for genotypes were achieved under conditional logistic regression models adjusted for age, gender, smoking, cigarette/tobacco smoking, and alcohol consumption. Logistic regression analyses, adjusting for gender, exposure time, cigarette smoking, and alcohol consumption, were conducted to address potential sources of bias. Differences in allele-specific promoter activity and gene expression were compared by Student's or paired t-tests, as appropriate. Haplotype analysis of the polymorphisms was performed using the SHEsis platform. All *P* values were corrected (*P*c) with Bonferroni's corrections and $P < 0.05$ indicates statistical significance.

Results

Demographic characteristics of the study subjects and Hardy-Weinberg testing for selected SNPs

General characteristics (gender, cigarette smoking, alcohol consumption, and exposure time to lead) and BLLs of the lead poisoning cases and controls are shown in **Table 1**. There were no significant differences in general characteristics between the two groups ($P > 0.05$). The BLL did differ significantly, with mean values higher in cases (513.57 ± 5.97 μg/L) than in controls (109.84 ± 3.08 μg/L) ($P < 0.001$).

General information regarding selected SNPs and the Hardy-Weinberg test results are shown

Table 3. Distribution of three polymorphisms and the association with lead poisoning

Gene	Genetic models	Genotypes	Cases		Controls		P^a	Adjusted OR
			n = 287	%	n = 355	%		(95% CI)[b]
CYP1A1	rs1048943							
	Codominant	GG	8	2.8	18	5.1	0.007	1.00 (Ref.)
		AG	107	37.3	167	47.0		1.78 (0.67-4.78)
		AA	172	59.9	170	47.9		3.03 (1.14-8.10)
	Dominant	GG/AG	115	40.1	185	52.1	0.002	1.00 (Ref.)
		AA	172	59.9	170	47.9		1.78 (1.25-2.54)
	Recessive	GG	8	2.8	18	5.1	0.145	1.00 (Ref.)
		AG/AA	279	97.2	337	94.9		2.37 (0.90-6.21)
	Alleles	G	123	21.4	203	28.6	0.003	1.00 (Ref.)
		A	451	48.6	507	71.4		1.58 (1.19-2.11)
CYP1B1	rs1056827							
	Codominant	GT	112	39.0	122	34.4	0.223	1.00 (Ref.)
		GG	175	61.0	233	65.6		0.76 (0.53-1.08)
	Alleles	G	462	80.5	588	82.8	0.282	1.00 (Ref.)
		T	112	19.5	122	17.2		1.25 (0.91-1.71)
CYP1B1	rs1056836							
	Codominant	GG	4	1.4	7	2.0	0.312	1.00 (Ref.)
		CG	49	17.1	76	21.4		0.91 (0.19-4.31)
		CC	234	81.5	272	76.6		1.47 (0.32-6.66)
	Dominant	GG/CG	53	18.5	83	23.4	0.130	1.00 (Ref.)
		CC	234	81.5	272	76.6		1.60 (1.04-2.47)
	Recessive	GG	4	1.4	7	2.0	0.575	1.00 (Ref.)
		CG/CC	283	98.6	348	98.0		1.33 (0.30-6.03)
	Alleles	G	57	9.9	90	12.7	0.124	1.00 (Ref.)
		C	517	90.1	620	87.3		1.50 (1.01-2.23)

[a]Two-sided X^2 test. [b]Adjusted for sex, exposure time, cigarette smoking, and alcohol consumption in logistic regression model.

in **Table 2**. rs1048943, rs1056827, and rs1056836 are missense mutations and χ^2 tests revealed that their minor allele frequencies (MAF) were all in the balance of Hardy-Weinberg ($P > 0.05$).

Multivariate analyses of CYP1A1 and CYP1B1 SNPs with the risk of lead poisoning

Three *CYP1A1* and *CYP1B1* SNPs were selected to genotype in 642 lead-exposed workers (287 lead poisoning cases and 355 controls). **Table 3** presents the genotype results and allele distributions of rs1048943, rs1056827, and rs1056836 in codominant, dominant, recessive, and allelic models. Results revealed that the genotype frequencies of rs1048943 in the codominant model among cases and controls were significantly different ($P = 0.007$). In the dominant and recessive models, rs1048943 AA genotype was significantly associated with increased lead poisoning risk ($P = 0.002$). Subsequent logistic regression analyses, adjusting for gender, exposure time, cigarette smoking, and alcohol consumption, were conducted to address potential sources of bias. Results showed that individuals with rs1048943 AA had increased lead poisoning risk, with an OR of 3.03 (95% CI = 1.14-8.10). In the allelic model, rs1048943 A (OR = 1.58, 95% CI = 1.19-2.11) allele conferred a significantly increased risk for lead poisoning ($P = 0.003$). Thus, present data revealed that the *CYP1A1* SNP, rs1048943, may have a significant association with lead poisoning susceptibility.

Association between the haplotypes of CYP1A1 and CYP1B1 SNPs with lead poisoning risk

Furthermore, analysis of the haplotype frequencies of the three SNPs was performed between

Table 4. Frequencies of inferred haplotypes among the cases and controls and their association with risk of lead poisoning

Haplotypes[a]	Case (n = 287*2)		Control (n = 355*2)		*P*[b]	OR (95% CI)	Global *P*[c]
	n	%	n	%			
AGC	321.13	55.9	392.15	55.2	0.960	0.994 (0.795-1.243)	< 0.001
AGG	43.01	7.5	42.64	6.0	0.323	1.247 (0.804-1.934)	
ATC	86.70	15.1	61.30	8.6	< 0.001	1.852 (1.308-2.623)	
GGC	88.18	15.4	121.88	17.2	0.322	0.860 (0.637-1.160)	
GGG	9.69	1.7	31.32	4.4	0.005	0.366 (0.176-0.759)	
GTC	20.99	3.7	44.66	6.3	0.028	0.556 (0.327-0.946)	
Others[d]	4.31	0.7	16.04	2.2		1.00 (Ref.)	

[a]The alleles of haplotypes were arrayed as rs1048943-rs1056827-rs1056836. [b]Two-sided X^2 test. [c]Generated by permutation test with 1,000 times of simulation. [d]Haplotypes with a frequency < 0.03 (ATG/GTG) were pooled into the mixed group.

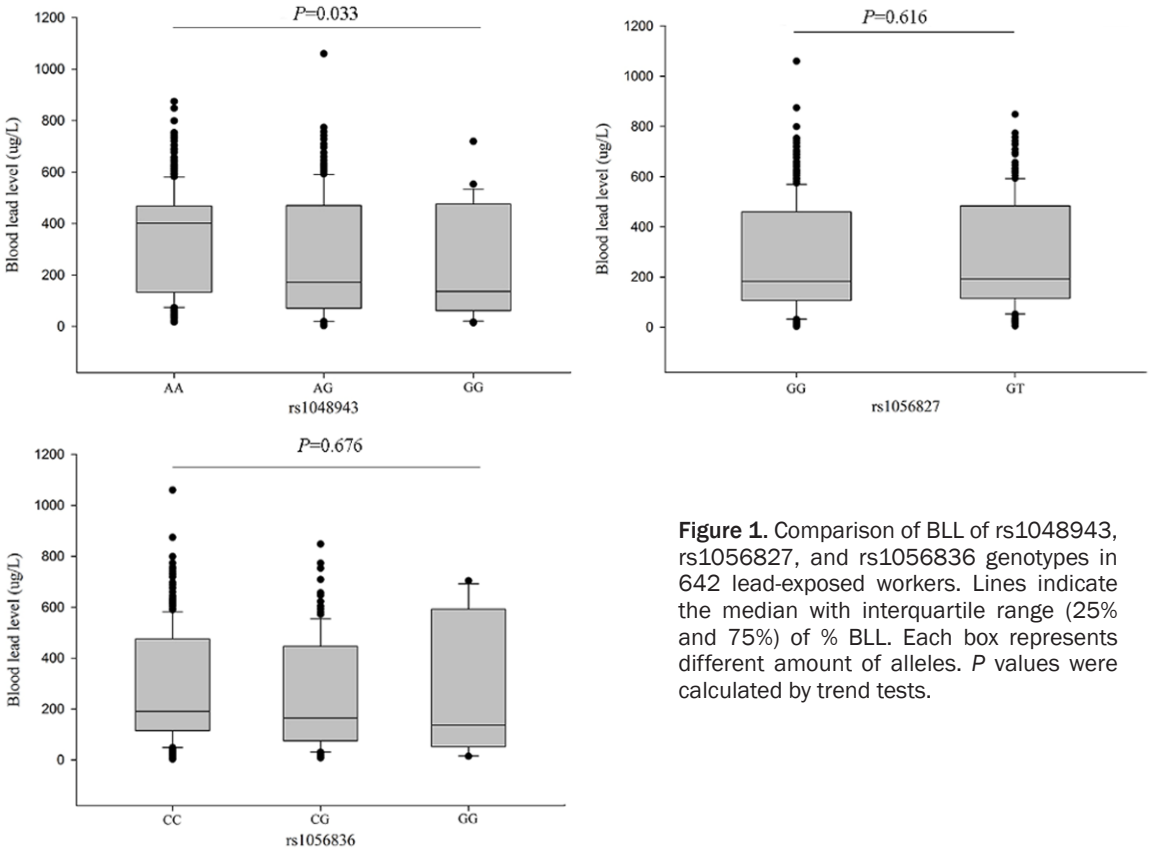

Figure 1. Comparison of BLL of rs1048943, rs1056827, and rs1056836 genotypes in 642 lead-exposed workers. Lines indicate the median with interquartile range (25% and 75%) of % BLL. Each box represents different amount of alleles. *P* values were calculated by trend tests.

cases and controls (**Table 4**). Six common haplotypes (frequency > 3%) derived from the three SNPs, accounting for 95% of the haplotype variations, were selected. The remaining haplotypes were pooled into the mixed group. Ultimately, haplotype ATC (rs1048943-rs1056827-rs1056836) was found to be associated with an increased risk of lead poisoning (OR = 1.852), while haplotypes GGG and GTC were linked to a decreased risk of lead poisoning (OR = 0.366 and 0.556, respectively).

Comparison of BLL of three SNPs genotypes

Figure 1 shows the results for the BLL of rs1048943, rs1056827, and rs1056836 genotypes in 642 lead-exposed workers. Subjects with the AA genotype of rs1048943 had a sig-

Table 5. MDR analysis results of the interaction between the three SNPs

Best model	Training balanced accuracy	Testing balanced accuracy	Cross-validation consistency	*P*	OR (95%CI)
rs1048943	0.5602	0.5602	10/10	0.0024	1.63 (1.19-2.23)
r1048943-rs1056827	0.5610	0.5431	8/10	0.0024	1.63 (1.19-2.23)
rs1048943-rs1056827-rs1056836	0.5671	0.5317	10/10	0.0009	1.70 (1.24-2.32)

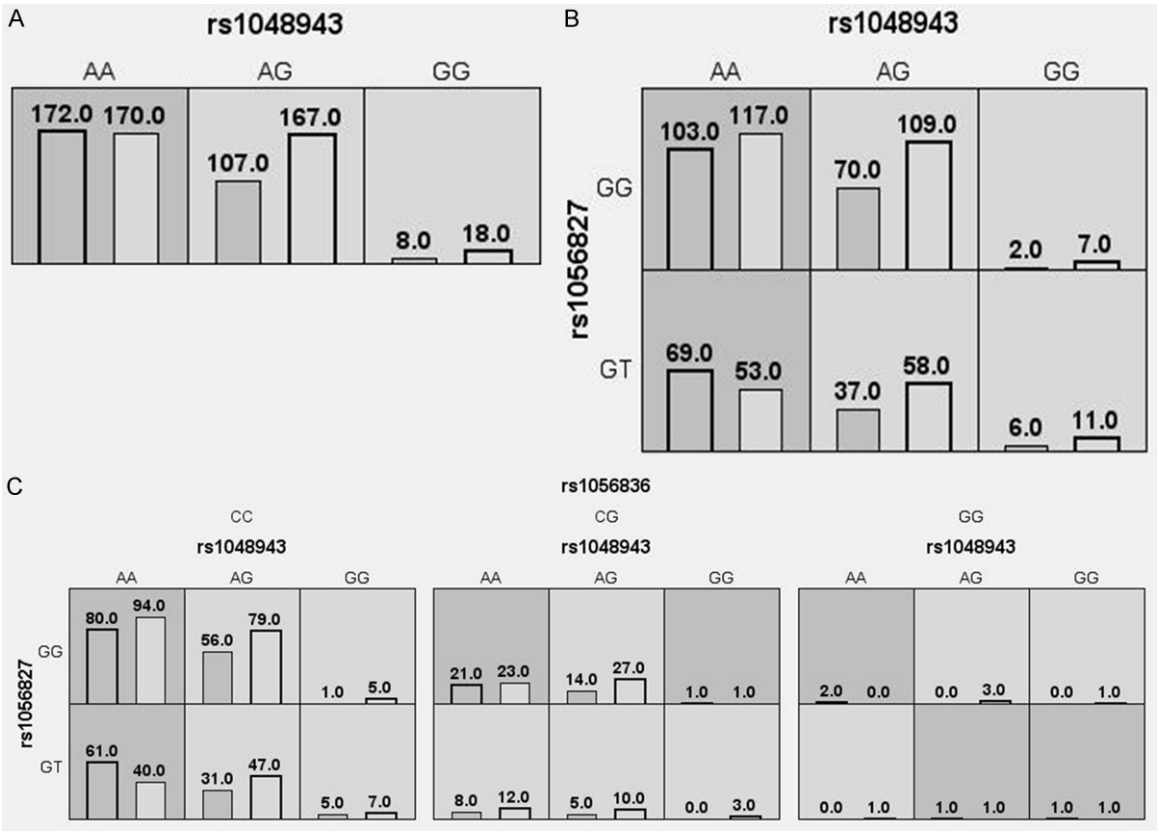

Figure 2. Graph model of the interaction between the three SNPs. The implications of bars and background color in each multifactor cell are as follows: Dark gray and light gray boxes present the high- and low-risk factor combinations, respectively. Left bars within each box represent cases while the right bars represented control. The heights of the bars are proportional to the sum of samples in each group. The multifactor cells labeled as "high risk" or "low risk" are then used to assess the classification and predication accuracy, thus identifying the best model in the subsequent steps.

nificantly higher BLL than people with the AG and GG genotype ($P = 0.0033$).

Multifactor dimensionality reduction (MDR) analyses of the interaction between the three SNPs

MDR analysis results of the interaction between the three SNPs are presented in **Table 5** and **Figure 2**. Interaction results suggested that the rs1048943-rs1056827 and rs1048943-rs1056827-rs1056836 model was related to increased lead poisoning risk (OR = 1.63 and 1.70, $P = 0.0024$ and 0.0009, respectively).

Discussion

Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variants in the human genome, with about fifteen million SNPs identified in humans [9]. Haplotypes, defined as specific sets of alleles observed on a single chromosome or on part of a chromosome, have been an integral part of human genetics for decades [10]. However, the

genomic distribution of SNPs is not homogenous. Most SNPs occur in noncoding regions of genes more frequently than in coding regions. At present, detection methods for SNPs include denaturing gradient gel electrophoresis (DDGE), single-strand conformational polymorphism (SSCP), cleaved amplified polymorphic sequence (CAPS), denaturing gradient gel electrophoresis (DDGE), and allele-specific PCR (such as TaqMan genotype-PCR).

In the current study, genetic association analysis was performed on three selected *CYP1A1* and *CYP1B1* SNPs (rs1048943, rs1056827, and rs1056836) in 287 lead poisoning cases and 355 controls by TaqMan genotyping assay. Results revealed that workers with the rs1048943 AA genotype or A allele of *CYP1A1* had a significantly higher risk of lead poisoning, with an OR of 3.03 and 1.58, respectively. Subsequent haplotype analysis showed that the haplotype ATC (rs1048943-rs1056827-rs1056836) conferred an increased risk of lead poisoning. These findings support the hypothesis that *CYP1A1* polymorphisms may contribute to susceptibility to lead poisoning in a Chinese population. To the best of our knowledge, this is the first association study showing that expression of a CYP gene correlates with risk of lead poisoning in a Chinese population.

It has been established that biotransformation plays an important role in the carcinogenic activity of environmental carcinogens. Large inter-individual variations in biotransformation have been reported and genetic polymorphisms in some xenobiotic-metabolizing and DNA repair enzymes, such as the CYPs can, in part, explain some of these differences. A study from Korashy and El-Kadi showed that lead significantly decreased TCDD-induced *CYP1A1* mRNA protein and catalytic activity levels in a concentration-dependent manner [4]. Importantly, this inhibition was specific to *CYP1A1* and not to other aryl hydrocarbon receptor (AhR)-regulated genes, as lead induced NAD(P)H: Quinone oxidoreductase 1 mRNA. As rs1048943 is a missense mutation in the *CYP1A1* gene, further research is required to determine whether it plays a role in susceptibility to lead poisoning via effects on *CYP1A1* activity.

The present study, however, had several limitations. First, while the sample size of this study was relatively large, compared to previous research, the power of the statistical tests may not be enough to detect small biological effects of individual SNPs. Therefore, a larger sample size is necessary in the future to confirm the effects of CYP polymorphisms on lead poisoning risk. Second, the subjects in this case-control study were limited to those in the Chinese population. Thus, present results may be more applicable to the Chinese Han population, with limited external generalizability.

Conclusion

A missense mutation (rs1048943) in the *CYP1A1* gene was found to be associated with susceptibility to lead poisoning, possibly contributing at a molecular level to the pathogenesis of this disease.

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Disclosure of conflict of interest

None.

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