Letter to the Editor

Association between the HOTAIR Polymorphism and Susceptibility to Lead Poisoning in a Chinese Population*

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This study explored the association between the lncRNA HOTAIR polymorphism and susceptibility to lead poisoning in a Chinese population. We speculated that lead poisoning caused elevated levels of oxidative stress, which, in turn, activate the HOTAIR gene to cause apoptosis. Three lncRNA HOTAIR tagSNPs (rs7958904, rs4759314, and rs874945) were genotyped by TaqMan genotyping technology in 113 lead-sensitive and 113 lead-resistant Chinese workers exposed to lead. Rs7958904 was significantly associated with susceptibility to lead poisoning (*P* = 0.047). The rs7958904 G allele had a protective effect compared with the C allele and reduced the risk of lead poisoning (*P* = 0.016). Rs7958904 may act as a potential biomarker for predicting the risk of lead poisoning and distinguishing lead-sensitive individuals from lead-resistant individuals.

Lead is an element widely used in various industries and a serious threat to human health. It can enter the human body *via* air, food, water, and dust, *via* the respiratory tract, digestive tract, and skin, and can significantly damage various systems and tissues. Acute and chronic exposure to lead causes irreversible toxicity in several human organs and systems, such as the nervous, hematopoietic, and reproductive systems, as well as the kidneys and bones[1]. Thus, there is an urgent need to explore valid biomarkers for predicting and preventing lead poisoning.

Because of the lack of an open reading frame of significant length, lncRNAs have no protein-coding capability. Studies have revealed that lncRNAs are involved in numerous cellular and tumor-related processes, including cell growth, transcriptional regulation, and tumorigenesis[2]. However, the functions of 99% of discovered lncRNAs remain unknown. The abnormal expression of HOTAIR in many cancers, such as lung, breast, liver, and ovarian cancers, illustrates that HOTAIR may play an important role in carcinogenesis and may be a potential tumor biomarker.

Our pioneering studies have shown that susceptibility to lead poisoning might be associated with the δ-ALAD, VDR, and HFE genes and that polymorphisms among these genes might influence the bioaccumulation and toxic effects of lead[3]. Lead poisoning has been recognized as a multifactorial disease, and dysregulation of oxidative stress is acknowledged as a crucial pathogenic factor for this disease. Recent studies have reported that lncRNA HOTAIR is involved in the altered oxidative stress level, cell proliferation, cell cycle progression, and apoptosis[4]. Studies have also revealed that aberrant expression of HOTAIR may cause cell apoptosis in tumors via oxidative stress[5]. Considering the roles of lncRNA HOTAIR in oxidative stress and cell apoptosis, we speculate that HOTAIR polymorphisms may be associated with susceptibility to lead poisoning.

A total of 1,130 workers employed in the same type of work were selected randomly from different lead-acid battery enterprises in Jiangsu Province, China. After matching external lead exposure levels and working age with lead exposure, 113 workers (10%) with the highest blood lead levels (BLLs) were selected as the lead-sensitive group, while another 113 workers (10%) with the lowest BLLs were

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selected as the lead-resistant group. All workers started their lead-related work after an orientation health check. All workers were initially healthy without aberrant BLLs. All workers were required to fill out a standard questionnaire, which collected information about demographic characteristics, occupational history, family history, medical history, individual habits, and self-described symptoms. This study was approved by the Ethics Committee of the Jiangsu Province Center for Disease Control and Prevention (No. 2015025, 18 July 2012), and informed consent was obtained from each worker.

Venous blood (5 mL) was collected into metal-free vacuum blood collection tubes, placed on ice in a portable refrigerator, and transported to the freezer in our laboratory. A 0.2% nitric acid solution was added to the blood samples for further reaction, which was necessary before detection. BLLs were measured by PE900T atomic absorption spectrometer within 48 h of collecting the samples. According to the Chinese guidelines, the GBW09139h-09140h and GBW (e) 09054b-09056b standards were used to measure control BLLs. Each measurement was repeated by three people independently in a blinded fashion, and BLLs of samples were considered to be genuine when the concentration errors were < 5%.

Venous blood (5 mL) was collected into an anti-coagulation tube with EDTA and centrifuged immediately at 3,000 ×*g* for 5 min to separate the plasma. DNA was extracted from the plasma, which was separated using QIAcube HT Plasticware and the QIAamp 96 DNA QIAcube HT Kit (Qiagen, Dusseldorf, Germany) according to the manufacturer instructions and stored at -80 °C. The Nanodrop One^C ultramicro ultraviolet spectrophotometer was used to check the purity of DNA (Thermo Scientific, Waltham, MA, USA).

TagSNPs in the HOTAIR genes were selected based on the HapMap database, the NCBI database, and a literature review. Single nucleotide polymorphisms (SNPs) were finally selected according to the criteria of minor allele frequency > 0.05 in a Chinese population (the sixth exon region: rs7958904; the first intron region: rs4759314; 3'-flanking region: rs874945). Genotypes of the three selected SNPs (rs7958904, rs4759314, and rs874945) were screened by ABI TaqMan SNP genotyping assays (Applied Biosystems, Foster City, CA, USA). The extracted DNA and genotyping assay mixtures were added to TaqMan universal PCR master mix (Roche, Branchburg, NJ, USA) per the manufacturer's recommendations. The genotyping procedures were performed using the ABI 7900 real-time PCR system (Applied Biosystems). The data were analyzed with ABI 7900 System SDS2.4 software. The SNP primer sequences are listed in Table 1, and the fluorescent probe sequences are listed in Table 2.

SPSS 23.0 software (SPSS Inc., Chicago, IL, USA) was used for the statistical analysis. The Hardy-Weinberg equilibrium was checked by the goodness-of-fit χ^2 test between the lead-sensitive and lead-resistant groups (P > 0.05). Categorical variables are presented as percentages, and continuous variables are described as mean ± standard deviation. Student's *t*-test was applied to differentiate the two groups for age and BLLs, while differences in individual characteristics were compared by Pearsons χ^2 test. A conditional logistic regression model was adopted by adjusting for sex, age, smoking, drinking, and eating or drinking in the workplace to calculate odds ratios (*OR*s) and 95% confidence intervals (95% *CI*s) for the different genotypes. All significance tests were two-tailed (α = 0.05), and P-values < 0.05 were considered significant.

The characteristics and BLLs of the 113 lead-sensitive and 113 lead-resistant workers are presented in Table 3. No significant differences were found between the lead-sensitive and lead-resistant groups for sex (P = 0.506), smoking (P = 0.246), education (P = 0.412), drinking (P = 0.080), or eating or

Table 1. SNPs PCR Primers Sequences

Locus	Primers Sequence (5'-3')
rs4759314	F: ATGATCTGCTTGGAAGGGATATAAA
	R: GCCTGGGCTTTTTCAGGTTT
rs874945	F: GGCCAGCGCCTCAGACT
	R: GCTCGTCCCTGAGCACCCTA
rs7958904	F: TGAACGCCCAGAGAACGCT
	R: ATGGAGATGATAAGAAGAGCAAGGAA

Table 2. SNPs Fluorescent Probes Sequences

Probes Name	Probes Sequence (5'-3')
rs4759314-P-A	FAM-AGGCCAAGCGGAT-MGB
rs4759314-P-G	HEX-AGGCCAGGCGGAT-MGB
rs874945-P-A	FAM-CTCTTGGAAGTCTC-MGB
rs874945-P-G	HEX-CTCTTGGAGGTCTC-MGB
rs7958904-P-C	FAM-CGGCTCCGGTCAG-MGB
rs7958904-P-G	HEX-CGGCTCGGGTCAG-MGB

drinking in the workplace (P = 0.847). The average age of individuals in the lead-sensitive group (38.39 ± 8.85 years) was slightly higher than that in the lead-resistant group (35.86 ± 10.26) (P = 0.047). BLLs of the lead-sensitive group (top 10% of BLLs) and the lead-resistant group (bottom 10% of BLLs) were 513.52 ± 63.86 and 89.34 ± 15.39 μg/L, respectively (P < 0.001).

Three HOTAIR tagSNPs were selected for genotyping by TaqMan SNP genotyping assays. Table 4 shows the genotype and allele distributions of rs7958904, rs4759314, and rs874945 in the lead-sensitive and lead-resistant groups. An allelic association analysis revealed that only rs7958904 was significantly associated with susceptibility to lead poisoning (P = 0.047). The rs7958904 G allele was more frequent in the lead-resistant group than in the lead-sensitive group, and the difference was significant (16.81% *vs*. 26.11%, P = 0.016, *OR* = 0.56, 95% *CI* = 0.35-0.90). A logistic regression analysis revealed that workers with the rs7958904 CG/GG genotype had a decreased lead poisoning risk with an *OR* of 0.49 (P = 0.013, 95% *CI* = 0.28-0.86). These results suggest that the rs7958904 G allele conferred protective effects compared with the C allele and suggest that workers with the G allele had a reduced risk of lead poisoning.

We further examined the effect of different rs7958904 genotypes on a series of risk characteristics in a dominant model of lead poisoning. The results are summarized in Table 5. Significant differences were found in age and smoking habits between the CC and CG/GG genotypes (P < 0.01); differences were observed in education, drinking, and eating or drinking habits in the workplace (P < 0.05). Workers < 35 years old who carried the rs7958904 CG/GG genotype had a decreased risk of lead poisoning when compared with those carrying the CC genotype (P = 0.008, *OR* = 0.31, 95% *CI* = 0.13-0.74). After normalization for smoking and drinking, the risk of lead poisoning in workers with the CG/GG genotype was reduced in the nonsmoking group (P = 0.007, *OR* = 0.38, 95% *CI* = 0.19-0.78) and no drinking group (P = 0.025, *OR* = 0.47, 95% *CI* = 0.24-0.91). These decreased risks were distinctly found among literate workers carrying the CG/GG genotype with education up to the lower secondary level (P = 0.027, *OR* = 0.20, 95% *CI* = 0.05-0.83). The risk of lead poisoning remained low with occasional eating or drinking in the workplace, with an *OR* of 0.23 (P = 0.011, 95% *CI* = 0.08-0.71) in workers with the CG/GG genotype.

In our study, we discovered that the HOTAIR gene rs7958904 polymorphism was significantly associated with susceptibility to lead poisoning. The results also suggest that the rs7958904 G allele conferred protective effects when compared with the C allele. In addition, one study indicated that rs7958904 is significantly associated with susceptibility to cervical cancer, and that subjects with

Table 3. Characteristics of the 10% Most Lead-sensitive and 10% Most Lead-resistant Groups

Characteristics	Group, *n* (%)		*P*
	Lead-sensitive (*n* = 113)	Lead-resistant (*n* = 113)	
Gender			0.506
Male	57 (50.4)	52 (46.0)	
Female	56 (49.6)	61 (54.0)	
Age (years)	38.39 ± 8.85	35.86 ± 10.26	0.047*
Smoking			0.246
No	75 (66.4)	83 (73.4)	
Yes	38 (33.6)	30 (26.6)	
Education			0.412
Literate and up to lower secondary level	26 (23.0)	21 (18.6)	
Low up to middle secondary level	87 (77.0)	92 (81.4)	
Drinking			0.080
No	82 (72.6)	93 (82.3)	
Yes	31 (27.4)	20 (17.7)	
Eat or drink in workplace			0.847
No	30 (26.6)	31 (27.4)	
Occasionally	39 (34.5)	35 (31.0)	
Yes	44 (38.9)	47 (41.6)	
Mean ± SD	513.52 ± 63.86	89.34 ± 15.39	< 0.001*

Note. *P value of two-sided student's t-test for age and blood lead level.

the rs7958904 CC genotype have an increased risk of cervical cancer compared with those with the GG/GC genotype[6]. Therefore, the G allele was a protective factor when compared with the C allele. Furthermore, a significant association has been reported between the rs7958904 SNP and reduced risk of colorectal cancer cell proliferation[7]. These findings support our result that the HOTAIR polymorphism may contribute to susceptibility to lead poisoning. To our knowledge, this may be the first study showing that the rs7958904 polymorphism in the HOTAIR gene is associated with a lower risk of lead poisoning in a Chinese population. HOTAIR is involved in alterations of cell proliferation, cell cycle progression, and cell apoptosis. Some studies have reported that high expression of HOTAIR facilitates tumor tissue growth, while knocking out HOTAIR may affect the cell cycle and promote apoptosis *via* a blocking effect on cell growth[8]. Some studies have found the LncRNA HOTAIR is involved in changing the oxidative stress level, cell proliferation, cell cycle progression, and apoptosis[9]. Other studies suggest that aberrant expression of HOTAIR may cause cell apoptosis in tumors via cellular oxidative stress[5].

Several studies have shown that lead toxicity is

Table 4. Genotype and Allele Distributions of HOTAIR SNPs in Lead-sensitive Group and Lead-resistant Group

Genotype	Lead-sensitive (*n* = 113)		Lead-resistant (*n* = 113)		HWE	*P**	Adjusted *OR* (95% *CI*)*
	n	%	*n*	%			
rs7958904					0.732		
CC	79	69.91	61	53.98			1.00 (Ref.)
CG	30	26.55	45	39.82		0.023	0.50 (0.28-0.91)
GG	4	3.54	7	6.20		0.200	0.41 (0.11-1.59)
CG/GG	34	30.09	52	46.02		0.013	0.49 (0.28-0.86)
CC/CG	109	96.46	106	93.80			1.00 (Ref.)
GG	4	3.54	7	6.20		0.299	0.49 (0.13-1.88)
C allele	188	83.19	167	73.89			1.00 (Ref.)
G allele	38	16.81	59	26.11		0.016	0.56 (0.35-0.90)
P trend						0.047	
rs4759314					0.535		
AA	95	84.07	98	86.73			1.00 (Ref.)
AG	15	13.27	14	12.39		0.575	1.26 (0.56-2.80)
GG	3	2.66	1	0.88		0.307	3.31 (0.33-32.89)
AG/GG	18	15.93	15	13.27		0.386	1.40 (0.66-3.00)
AA/AG	110	97.34	112	99.12			1.00 (Ref.)
GG	3	2.66	1	0.88		0.320	3.20 (0.32-31.73)
A allele	205	90.71	210	92.92			1.00 (Ref.)
G allele	21	9.21	78	7.08		0.286	1.43 (0.74-2.74)
P trend						0.423	
rs874945					0.777		
GG	83	73.45	72	63.72			1.00 (Ref.)
AG	24	21.24	37	32.74		0.072	0.56 (0.30-1.05)
AA	6	5.31	4	3.54		0.444	1.69 (0.44-6.53)
AG/AA	30	26.55	41	36.28		0.167	0.66 (0.37-1.19)
GG/AG	107	94.69	109	96.46			1.00 (Ref.)
AA	6	5.31	4	3.54		0.326	1.96 (0.51-7.49)
G allele	190	84.07	181	80.09			1.00 (Ref.)
A allele	36	15.93	77	19.91		0.435	0.83 (0.51-1.34)
P trend						0.289	

Note. *Adjusted for gender, age, smoking, drinking and eat or drink in workplace.

related to oxidative stress because it generates reactive oxygen species (ROS), interferes with antioxidant enzyme activities, and breaks the balance in the pro-oxidant/antioxidant defense system, resulting in oxidative damage to proteins, nucleic acids and lipid compounds, cell apoptosis and necrosis, and metabolic disorders of tissues and organs of humans, as well as chronic diseases, such as cancer[10]. Once the equilibrium state of oxidative stress is broken, it promotes apoptosis and causes pathological damage. Lead poisoning is associated with oxidative stress and cell apoptosis[1]. Lead participates in the Fenton reaction to produce more ROS, which directly induces oxidative stress[9]. Lead easily covalently binds to the body's antioxidants, such as reduced glutathione (GSH), thereby depriving it of ROS abatement[9]. In addition, lead interacts with antioxidant enzymes and GSH-related enzymes, affecting enzyme activities and concentrations, and interfering with the body's redox reactions[10]. Exposure to lead may cause oxidative stress, and produce active oxygen free radicals (ROS), which promote the action of apoptotic mediators or transmitters in many tissue systems. Thus, we speculate that lead poisoning causes elevated levels of oxidative stress, which, in turn, activate the HOTAIR gene to cause apoptosis.

In summary, our study provides new evidence that individuals possessing the G allele in HOTAIR tagSNP rs7958904 were at a decreased risk of lead poisoning. Thus, the G allele may confer a protective effect to workers upon lead exposure when compared with the C allele. Rs7958904 may act as a potential biomarker for predicting the risk of lead poisoning and distinguishing lead-sensitive from lead-resistant individuals. However, the current study had several limitations. The statistical tests may not be conclusive due to the lower biological effects of an isolated SNP. Further studies are required to confirm our findings with larger sample sizes and in diverse ethnic populations.

Table 5. Stratified Analysis of rs7958904 Polymorphism and Lead Poisoning

Characteristics	CC (Lead-sensitive /Lead-resistant)		CG/GG (Lead-sensitive /Lead-resistant)		*P**	Adjusted *OR* (95%*CI*)*
	n	(%)	*n*	(%)		
Gender						
Male	39/30	35.78/27.52	18/22	16.51/20.18	0.377	0.68 (0.29-1.60)
Female	40/31	34.19/26.50	16/30	13.68/25.64	0.057	0.46 (0.21-1.02)
Age (years)						
≤ 35	36/32	31.58/28.07	12/34	10.53/29.82	**0.008**	**0.31 (0.13-0.74)**
> 35	43/29	38.39/25.89	22/18	19.64/16.07	0.591	0.80 (0.35-1.81)
Smoking						
No	53/42	33.54/25.58	22/41	13.92/25.95	**0.007**	**0.38 (0.19-0.78)**
Yes	26/19	38.24/27.94	12/11	17.65/16.18	0.622	0.77 (0.27-2.20)
Education						
Literate and up to lower secondary level	19/7	40.43/14.89	7/14	14.89/29.79	**0.027**	**0.20 (0.05-0.83)**
Low up to middle secondary level	60/54	33.52/30.17	27/38	15.08/21.23	0.194	0.66 (0.35-1.24)
Drinking						
No	59/49	33.71/28.00	23/44	13.14/25.14	**0.025**	**0.47 (0.24-0.91)**
Yes	20/12	39.22/23.53	11/8	21.57/15.69	0.988	1.01 (0.29-3.55)
Eat or drink in workplace						
No	18/17	29.51/27.87	12/14	19.67/22.95	0.924	1.06 (0.33-3.35)
Occasionally	31/17	41.89/22.97	8/18	10.81/24.32	**0.011**	**0.23 (0.08-0.71)**
Yes	30/27	32.97/29.67	14/20	15.38/21.98	0.406	0.68 (0.28-1.68)

Note. *Adjusted for sex, age, smoking, education, drinking, eat or drink in workplace.

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