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Interplay analysis of lead exposure with key cardiovascular gene polymorphisms on blood pressure in a cross-sectional study of occupational workers

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An increasing number of studies have shown that lead is an important cardiovascular risk factor, but the impact of cardiovascular related gene polymorphisms on lead induced cardiovascular diseases is still unclear. To assess the interaction of lead exposure and related key cardiovascular regulating gene polymorphisms on blood pressure traits, three single-nucleotide polymorphisms including *NOTCH1* rs3124591, Cerebral cavernous malformations 3 (*CCM3*) rs3804610 and Vascular endothelial growth factor receptor type 2 (*VEGFR2*) rs2305948 were selected and genotyped using improved multiplex ligase detection reaction method in 568 lead exposure workers in South China. General characteristics, blood lead and biochemical parameters including glucose, lipid profile and creatinine were also collected according to standard protocols. Regression analysis was used to evaluate the association of blood pressure with lead exposure, polymorphisms and their interaction. This study displayed that *CCM3* rs3804610 had a positive interaction with lead and *VEGFR2* rs2305948 had a negative interaction with lead. Specifcally, compared with the wild-type population, the blood lead of the genotype population carrying the risk allele increased by 1 μg/dL, systolic blood pressure increased by 0.53 mmHg ($p < 0.01$) and diastolic blood pressure increased by 0.34 mmHg ($p < 0.05$) for *CCM3* rs3804610, and systolic blood pressure decreased by 0.28 mmHg ($p < 0.05$) and diastolic blood pressure decreased by 0.22 mmHg ($p < 0.05$) for *VEGFR2* rs2305948. Thus our findings showed that the interaction between *CCM3* rs3804610 and *VEGFR2* rs2305948 and lead exposure were associated with blood pressure and may provide guidance for future research on hypertension prevention and personalized clinical treatment in lead exposed populations.

Keywords Lead exposure, blood pressure , Cardiovascular regulating gene , Single-nucleotide polymorphism , Gene-environment interaction

Lead is a common toxic metal element that is widely present in our living or working environment. Due to its widespread application, lead pollution has become a global problem[1]. In China, occupational exposures in industries that still use lead, such as mining, smelting, and recycling, pose a significant risk to workers.The hazard of lead to the human body is multifaceted, among which cardiovascular diseases (CVDs) are particularly prominent[2]. A systematic review and meta-analysis of 37 epidemiological studies, including prospective cohorts, case-control, and nested case-control studies, found a positive correlation between environmental lead exposure and CVD risk[3]. A study on the health impact model of lead on cardiovascular diseases in the United States

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reported that approximately 16–46% of the decline in CVD-related mortality from 1999 to 2014 could be attributed to the reduction in blood lead levels[4].

Hypertension plays an important role in CVDs. Long term high blood pressure can lead to changes in the structure and function of the heart and blood vessels, thereby increasing the risk of CVDs[5]. In recent years, the relationship between lead and hypertension has garnered significant attention. However, epidemiological studies investigating the associations between lead exposure and hypertension among occupationally exposed workers are relatively limited. In a study involving 395 molybdenum miners and iron and steel foundry workers, Lu Shi et al. observed that increased prevalence ratios for hypertension among the quartile of urinary concentrations of lead were positive[6]. Lamas et al. showed that systolic blood pressure (SBP) and diastolic blood pressure (DBP) increased in line with a 1 μg/dL increase in blood lead level (BLL), and a BLL ≥ 6.87 μg/dL was associated with hypertension in lead-exposed workers in the Republic of Korea[7]. In a repeated-measure study, Han et al. found that the average annual increase of SBP or DBP showed an upward trend in different Pb dose groups among lead-exposed workers in China[8]. Therefore, further studies are necessary to gain a deeper understanding of this relationship in lead-exposed workers.

Whole genome association studies (GWAS) have made significant progress in the genetic basis of complex diseases such as hypertension, but this approach also has limitations[9]. An important point is that the design of GWAS usually does not directly consider the impact of environmental factors[10]. Therefore, although GWAS may reveal some gene regions associated with disease risk, it may overlook genes that are sensitive to environmental factors such as lead exposure. A recent study confirmed the importance of the aminolevulinate dehydratase (*ALAD*) gene in lead kinetics even at low exposure levels[11]. Nunes et al. observed that polymorphisms related to DNA repair pathways may modulate lead-induced DNA damage[12]. Besides, the association between lead exposure and lipid profile, and associated polymorphisms were reported[13]. Although the above studies may have indirect implications for cardiovascular research, including hypertension, the direct association between key cardiovascular genes and lead exposure is still limited.

Notch homolog 1 (*NOTCH1*), cerebral cavernous malformations 3 (*CCM3*) and vascular endothelial growth factor receptor type 2 (*VEGFR2*) genes are key cardiovascular genes, playing important roles in the normal development and function of the cardiovascular system[14–16]. The abnormal expression or dysfunction of them may lead to the occurrence and development of cardiovascular and cerebrovascular diseases[17–19]. On the other hand, we and other researchers found that *CCM3* and VEGF signaling played a crucial role in lead induced vascular toxicity, especially in angiogenesis, which is observed in many diseases, such as atherosclerosis, hypertension[20–22]. Notch signalingis regarded as a master regulator of angiogenesis and vascular remodeling. The Notch1 signaling acts to suppress the expression of VEGFR2 in adjacent endothelial cells, resulting in a situation where a single cell exhibits elevated levels of VEGFR2 compared to its neighboring cells, thereby making it highly sensitive to VEGF[23]. However, there is currently a lack of in-depth understanding of the role of *NOTCH1* in lead-induced cardiovascular toxic effects.

In a previous study, we assessed *CCM3* genetic polymorphismin arsenic exposed workers and observed that interactions between rs3804610 and arsenic exposure boosted the hazard of increased systolic pressure[24]. Mohamed et al. performed a systematic mutation-analysis based on DNA-sequencing of all coding exons and adjacent splice consensus sequences of *NOTCH1* gene, they demonstrated that Notch1 rs3124591 play a role in bicuspid aortic valve[25]. Several studies explored the potential relationships between polymorphisms in *VEGFR2* and coronary heart disease or major adverse cardiac events, consistent results were found for rs2305948 polymorphism[26,27]. However, investigations on whether these genetic polymorphisms affect the association between lead exposure and hypertension remains unexplored. In this study, We investigated the genotype distribution characteristics of *NOTCH1*, *CCM3* and *VEGFR2* polymorphisms (rs3124591, rs3804610, rs2305948) in lead exposed populations and evaluated the impact of lead exposure and polymorphisms interaction on blood pressure. The findings may provide possible personalized treatments guideline for hypertension in lead-exposed populations.

Results

Basic characteristics of the population

Among a total of 568 workers, the blood lead level ranged from 10.37 μg/L to 864.07 μg/L, with an average of 72.89 μg/L (SD = 88.48). There were 475 male workers (83.63%). Current smokers accounted for 37.15%. Drinkers accounted for 21.48%. According to China's body mass index (BMI) standard definition, over one-fifth of people were overweight (BMI above 24). Table 1 shows that the age, BMI, total cholesterol (TC), triglyceride (TG), lowdensity lipoprotein cholesterol (LDL-c), fasting blood glucose (FBG), and BLL of the high lead group were significantly higher than those of the low lead group. In addition, the high lead group had more smokers and drinkers. In terms of blood pressure, the high lead group showed a significant increase in SBP and DBP.

Distribution characteristics of polymorphic genotypes in different lead exposed workers

Polymorphism analysis showed that the *NOTCH1* rs3124591 locus did not conform to the Hardy-Weinberg equilibrium (HWE) ($p < 0.05$) in the overall population, but conforms to the HWE ($p > 0.05$) in the low lead exposure population. The *CCM3* rs3804610 locus and *VEGFR2* rs2305948 locus in the total population conformed to HWE ($p > 0.05$). There was no statistically significant difference in the distribution of genotypes between low lead exposure and high lead exposure workers (Table 2).

The difference of basic characteristics among workers with different polymorphic genotypes

Table 3 shows the basic characteristics of the population among different gene single nucleotide polymorphisms (SNPs) and genotypes. The genotype of *NOTCH1* rs3124591 carrying the risk allele had a lower proportion in males compared to the wild type, with higher BMI, TC, and blood lead, but the difference was not statistically

	N (%), Mean ± SD			
	Overall	**Low lead exposure**	**High lead exposure**	***P*-value**
Age	31.88 ± 10.55	25.32 ± 5.606	38.41 ± 10.264	<0.001
Sex (male)	475(83.63)	217(76.40)	258(90.85)	<0.001
Years of work	9.12 ± 10.91	2.37 ± 3.687	15.92 ± 11.538	<0.001
Smoking (Yes)	211(37.15)	69(24.30)	142(50.00)	<0.001
Drinking (Yes)	116(21.48)	42(14.79)	74(26.06)	0.001
BMI(kg/m^2)	22.06 ± 3.78	20.80 ± 2.98	23.30 ± 4.07	<0.001
BMI ≥ 24	135(23.77)	37(13.03)	98(34.50)	<0.001
TC(mmol/L)	4.85 ± 1.14	4.62 ± 1.08	5.09 ± 1.15	<0.001
TG(mmol/L)	1.26 ± 1.06	1.00 ± 0.70	1.53 ± 1.28	<0.001
LDL-c(mmol/L)	2.31 ± 1.09	1.88 ± 0.82	2.73 ± 1.15	<0.001
HDL-c(mmol/L)	1.53 ± 1.01	1.53 ± 0.27	1.53 ± 1.24	0.997
SCr(μmol/L)	79.84 ± 54.27	77.41 ± 21.93	82.28 ± 73.61	0.311
FBG(mmol/L)	4.07 ± 1.34	3.93 ± 1.11	4.21 ± 1.52	0.017
SBP(mmHg)	122 ± 14	120 ± 12	123 ± 15	0.005
DBP(mmHg)	78 ± 10	77 ± 9	80 ± 10	<0.001
BLL(μg/L)	72.89 ± 88.48	25.58 ± 5.80	120.21 ± 105.63	<0.001

Table 1. General characteristics of study participants ($N = 568$). BMI, body mass index ; TC, total cholesterol; TG, triglyceride;LDL-c, low density lipoprotein cholesterol; HDL-c, high density lipoprotein cholesterol; SCr, serum creatinine; FBG, fasting blood glucose; SBP, systolic blood pressure; DBP, diastolic blood pressure; BLL, blood lead level. *P* values were calculated by independent t test for continuous variables and chi-square test for categorical variables.

Gene polymorphism	*N* (%)			
	Overall	**Low lead exposure**	**High lead exposure**	***P*-value**
NOTCH1 rs3124591	567	284	283	0.320
TT	524(92.41)	260(91.55)	264(93.29)	
CT	39(6.88)	23(8.10)	16(5.65)	
CC	4(0.71)	1(0.35)	3(1.06)	
CCM3 rs3804610	567	284	283	0.932
TT	440(77.60)	220(77.46)	220(77.74)	
CT	120(21.16)	60(21.13)	60(21.20)	
CC	7(1.23)	4(1.41)	3(1.06)	
VEGFR2 rs2305948	566	284	282	0.274
CC	385(68.02)	200(70.42)	185(65.60)	
CT	164(28.98)	74(26.06)	90(31.91)	
TT	17(3.00)	10(3.52)	7(2.48)	
HWE test *P*-value				
NOTCH1 rs3124591	0.006	0.818	0.001	
CCM3 rs3804610	0.934	0.999	0.887	
VEGFR2 rs2305948	0.996	0.634	0.591	

Table 2. The distribution characteristics of related gene polymorphic genotypes in different lead exposed participants.

significant. The genotype of *CCM3* rs3804610 carrying risk alleles had a lower proportion of males and a higher proportion of smokers compared to the wild type, and the difference was statistically significant. Compared with the wild-type, the genotype carrying the risk allele *VEGFR2* rs2305948 had a higher proportion of drinkers and lower fasting blood sugar, and the difference was statistically significant; TC and blood lead level were higher, but the difference was not statistically significant.

The impact of the interaction between polymorphic loci of related genes and lead exposure on blood pressure

We next explored the interaction between blood lead and different SNPs.results of multiple linear regression, using SBP and DBP as dependent variables, adjusted for age, sex, BMI, smoking, alcohol consumption, TC, TG, LDL-c and FBG are shown in Table 4. The results showed that when SBP or DBP were used as the dependent

Variables	NOTCH1			CCM3			VEGFR2		
Mean ± SD or n(%)	TT	CT/CC	*P*-value	TT	CT/CC	*P*-value	CC	CT/TT	*P*-value
Age	31.89 ± 10.54	31.49 ± 10.66	0.813	31.91 ± 10.61	31.65 ± 10.37	0.805	31.45 ± 10.55	32.66 ± 10.50	0.204
Sex (male)	443(84.54)	31(72.09)	0.051	378(85.91)	96(75.59)	0.007	319(82.86)	154(85.08)	0.545
Years of service	9.19 ± 10.93	7.91 ± 10.55	0.461	9.23 ± 10.91	8.61 ± 10.89	0.569	8.80 ± 10.88	9.62 ± 10.90	0.403
Smoking (Non)	316(61.48)	30(69.77)	0.328	279(64.43)	67(54.03)	0.036	233(61.48)	113(63.84)	0.639
Drinking (Non)	402(79.13)	32(76.19)	0.694	337(79.11)	97(78.23)	0.900	306(82.04)	127(72.16)	0.010
BMI(kg/m^2)	21.98 ± 3.58	23.05 ± 5.65	0.074	22.11 ± 3.9	21.91 ± 3.35	0.610	22.22 ± 4.01	21.73 ± 3.23	0.159
TC(mmol/L)	4.84 ± 1.14	5.05 ± 1.17	0.263	4.84 ± 1.14	4.89 ± 1.15	0.723	4.80 ± 1.12	4.98 ± 1.18	0.085
TG(mmol/L)	1.25 ± 1.07	1.37 ± 0.98	0.505	1.28 ± 1.15	1.19 ± 0.67	0.398	1.26 ± 1.02	1.26 ± 1.16	0.994
LDL-c(mmol/L)	2.30 ± 1.10	2.41 ± 0.92	0.548	2.28 ± 1.10	2.40 ± 1.05	0.328	2.26 ± 1.09	2.41 ± 1.07	0.124
HDL-c(mmol/L)	1.53 ± 1.05	1.45 ± 0.50	0.709	1.54 ± 1.14	1.48 ± 0.29	0.663	1.55 ± 1.22	1.48 ± 0.37	0.558
SCr(μmol/L)	80.27 ± 55.92	74.42 ± 24.95	0.533	80.31 ± 60.23	78.28 ± 24.10	0.725	81.61 ± 64.57	76.18 ± 19.27	0.293
FBG(mmol/L)	4.07 ± 1.33	4.02 ± 1.51	0.824	4.03 ± 1.28	4.21 ± 1.54	0.182	4.16 ± 1.39	3.88 ± 1.22	0.026
SBP(mmHg)	122 ± 14	122 ± 11	0.994	121 ± 13	121 ± 15	0.714	122 ± 13	122 ± 14	0.864
DBP(mmHg)	78 ± 10	78 ± 8	0.797	78 ± 9	78 ± 11	0.537	78 ± 10	78 ± 10	0.617
BLL(μg/L)	71.64 ± 85.12	87.05 ± 123.26	0.273	75.2 ± 93.00	64.54 ± 70.62	0.232	68.46 ± 80	81.73 ± 104.19	0.096

Table 3. Descriptive characteristics among the different types of *NOTCH1* rs3124591, *CCM3* rs3804610 and *VEGFR2* rs2305948 SNPs.

	SBP			DBP		
Models	β	SE	*P*-value	β	SE	*P*-value
model1						
BLL	0.015	0.008	0.064	0.003	0.006	0.586
NOTCH1	0.604	2.837	0.832	2.090	1.982	0.292
NOTCH1×BLL	-0.007	0.021	0.729	-0.018	0.015	0.215
model2						
BLL	0.008	0.008	0.299	-0.003	0.006	0.600
CCM3	-2.690	1.918	0.161	-2.164	1.344	0.108
CCM3×BLL	0.053	0.020	0.008	0.034	0.014	0.014
model3						
BLL	0.028	0.010	0.006	0.011	0.007	0.107
VEGFR2	1.410	1.640	0.390	0.974	1.148	0.396
VEGFR2×BLL	-0.028	0.014	0.038	-0.022	0.010	0.025

Table 4. Multiple linear regression coefficients of SBP and DBP in different interaction models. β, regression coefficient; SE, standard error. All models were adjusted for age, years of work, sex, BMI, smoking, drinking, TC, TG, LDL-c and FBG. BLL: blood lead level.

variable, there were significant interactions between blood lead and *CCM3* rs3804610 and *VEGFR2* rs2305948 in Model 2 and Model 3, respectively ($P < 0.05$). In addition, the individual effect of blood lead on SBP in Model 3 was statistically significant ($P < 0.01$).

In Fig. 1A, compared with the wild-type population of *CCM3* rs3804610, the blood lead of the genotype population carrying the risk allele increased by 1 μg/dL, SBP increased by 0.53 mmHg. In Fig. 1B, compared with the wild-type population of *CCM3* rs3804610, the blood lead of the genotype population carrying the risk allele increased by 1 μg/dL, DBP increased by 0.34 mmHg. In Fig. 1C, compared with the wild-type population of *VEGFR2* rs2305948, the blood lead of the genotype population carrying the risk allele increased by 1 μg/dL, SBP decreased by 0.28 mmHg. In Fig. 1D, compared with the wild-type population of *VEGFR2* rs2305948, the blood lead of the genotype population carrying the risk allele increased by 1 μg/dL, DBP decreased by 0.22 mmHg.

Discussion

Industrial production is an important source of lead exposure, especially in industries such as mining, smelting, and paint manufacturing, causing serious impacts on the environment and worker health. The present study mainly found that the interaction between lead and *CCM3* rs3804610 and *VEGFR2* rs2305948 affected both SBP and DBP. Specifcally, *CCM3* rs3804610 had a positive interaction with lead and *VEGFR2* rs2305948 had a negative interaction with lead. Besides, this study also found that there were differences in basic characteristics such as sex, smoking, alcohol consumption, and FBG levels between population carrying mutated alleles and the wild-type population.

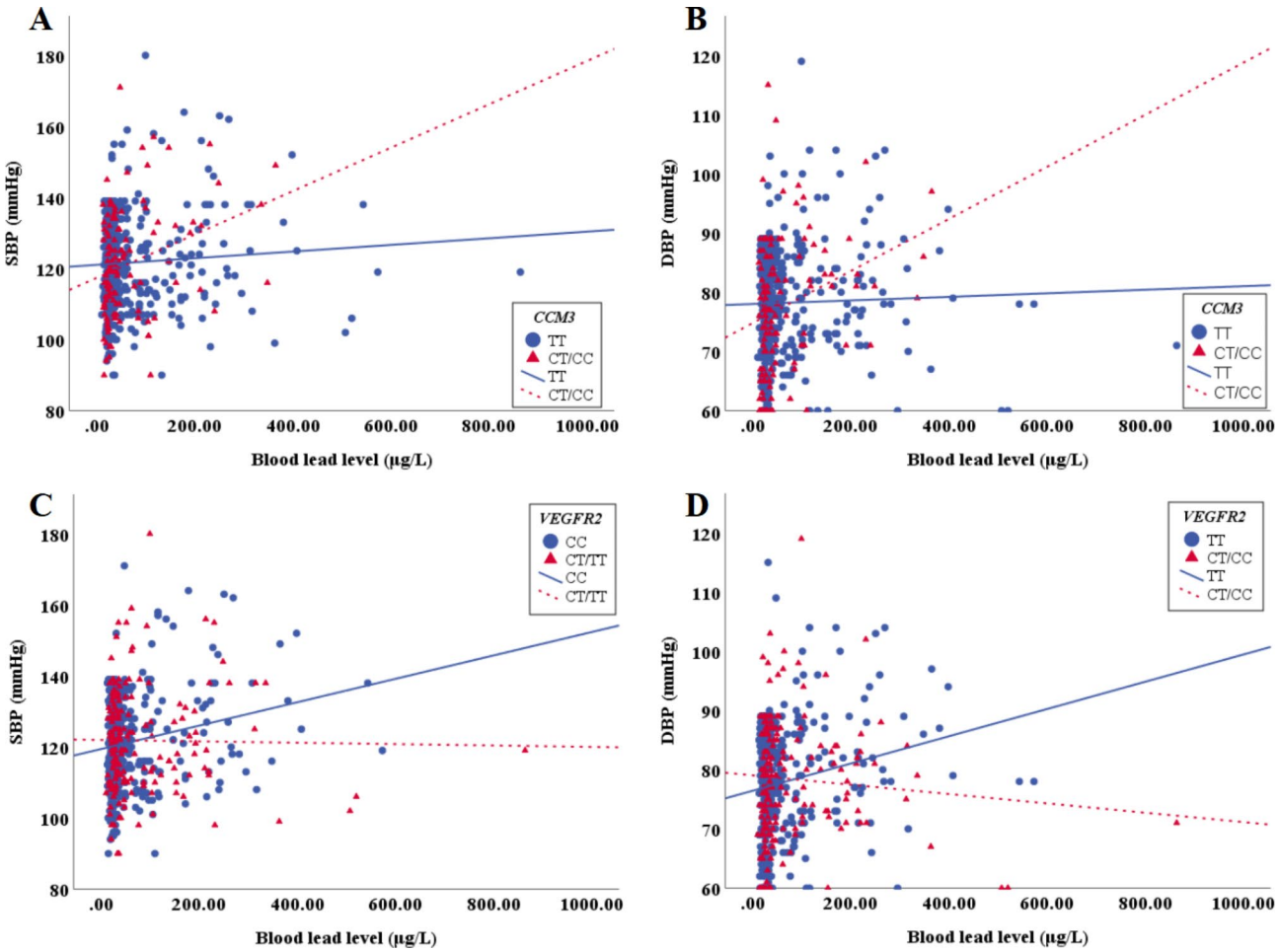

Fig. 1. The interaction effect of *CCM3* rs3804610 and *VEGFR2* rs2305948 on the levels of SBP and DBP in lead exposed workers. (**A**) *CCM3* rs3804610 and SBP; (**B**) *CCM3* rs3804610 and DBP; (**C**) *VEGFR2* rs2305948 and SBP; (**D**) *VEGFR2* rs2305948 and DBP.

The blood lead level in the low lead group is close to that of the general population in China in this study, while the high lead group significantly increases, but rarely exceeds 400 μg/L, which is the highest BLL acceptable for leadexposed workers in national and international criteria[28,29]. Thus, with the improvement of production technology and the emphasis on occupational health protection, the incidence of lead poisoning in the occupational population has significantly decreased. But this does not mean that there are no health risks associated with low-level lead exposure. When comparing blood pressure, it was observed that there was a significant increase in the high lead group. Other epidemiological studies have also shown an association between lead exposure and hypertension and the evidence is sufficient to infer the causal relationship[8,30–32]. Some studies using chronic exposure animal models have also shown that lead exposure is a risk factor for the development of hypertension[33–35]. These findings suggests that for lead-exposed workers, more frequent blood pressure monitoring and/or more aggressive antihypertensive treatment strategies may be needed to prevent the occurrence and development of CVDs. Future research should explore the potential impact of this discovery on clinical practice, including the development of more precise personalized treatment plans.

Many studies have confirmed that CVDs are complex and caused by both environmental and genetic factors. *NOTCH1*, *CCM3* and *VEGFR2* are key environmental response cardiovascular genes[21,28,36]. Detecting their genetic polymorphism may identify subsets of individuals who are more susceptible to the risk of exposure to harmful factors. Currently, there are few studies on the polymorphism of *NOTCH1* rs3124591 and *CCM3* rs3804610. Abramenko et al.[37] reported *NOTCH1* rs3124591 did not affect the risk of chronic lymphocytic leukemia and survival parameters of the patients. Another study on ductal breast carcinoma of the Han population of China revealed that the frequency of rs3124591 CT genotype was significantly higher in invasive ductal carcinoma and ductal carcinoma in situ patients than in usual ductal hyperplasia controls[38]. Our previous study suggest that arsenic exposure of population can assist *CCM3* polymorphism including rs3804610 in elevating SBP[24]. Several studies have found that *VEGFR2* polymorphism rs2305948 was associated with coronary heart disease and glioma risk[26,39,40]. In the present study, we firstly studied the frequency of these SNP genotypes in lead exposed populations. There was no statistically significant difference in the distribution of genotypes between low exposure and high exposure populations. When we observed the allele frequencies of these SNPs,

we identified *NOTCH1* rs3124591 was not consistent with Hardy-Weinberg equilibrium in high exposure populations, suggesting that the distribution of allele frequencies may be related with high lead exposure and requires validation from a larger sample size population.

In addition to studying the distribution differences of genetic polymorphisms between the low lead and high lead exposure populations, we also analyzed the impact of carrying risk alleles on the basic characteristics and health indicators in overall population. Mani et al.[41] reported that genes associated with lead metabolism *ALAD* polymorphism might contribute to increased susceptibility to high blood lead retention, and genotyping of ALAD in lead exposed subjects might be used as a prediction marker to impede tissue/organ damage due to lead toxicity. They recently found hemochromatosis(*HFE*) polymorphism might have a role in increasing the concentration of lead[42]. We found that workers carrying risk alleles had higher blood lead levels in *NOTCH1* rs3124591 and *VEGFR2* rs2305948 and had lower blood lead levels in *CCM3* rs3804610, suggesting a role in predisposition to lead and its associated effects. Further validation is needed due to the relatively small proportion of workers carrying risk alleles. When comparing blood pressure of different genotypes, no upward or downward trend was observed. This further indicates that these polymorphisms have little effect on blood pressure alone, and exploring their interaction with environmental factors can provide a deeper understanding of their role in CVDs.

Lim et al.[43] studied the impact of interaction between 42 GWAS SNPs and BMI, waist circumference, and drinking status on blood pressure in the Korean population with no interactions were found. Another study from Hollister et al. suggest a gene-environment interaction for blood pressure in African Americans[44]. Regression analysis of the present study observed that the effect of polymorphism or lead alone on blood pressure was not significant, but interestingly we identified *CCM3* rs3804610, *VEGFR2* rs2305948 interacted with lead to affect blood pressure, respectively. This may mean that they are interrelated in some way and have a common impact on blood pressure. Pathophysiological studies have extensively investigated the structural factor in hypertension, including large and small artery remodeling and functional changes[45]. Besides, our previous study found that CCM3 may regulate VEGFR2 receptor stability and association with apoptosis and endothelial cell growth[46]. DLL4-NOTCH and VEGF signaling are well-defined pathways for the regulation of vessel maturation and vessel permeability[47,48]. Lu et al.[49] found the inactivation of VEGFR2 signaling by Notch1 contributes to the delayed angiogenesis phenotype. Moreover, the loss of CCM3 impaired DLL4-Notch signaling disrupted the homeostasis of VEGF/VEGFR-2 pathway and activated Erk1/2, which was critical to the regulation of endothelial proliferation, migration, and sprouting[50]. Thus, further research is needed to elucidate whether the interaction between lead exposure and polymorphism on blood pressure is related to angiogenesis from the perspective of endothelial dysfunction. Additionally, exploring other genetic polymorphisms or evaluating the performance of longitudinal studies evaluating the evolution of hypertension is also beneficial in lead-exposed population.

This study provides evidence of the impact of key cardiovascular gene polymorphisms and lead interaction on blood pressure. However, some limitations of this study should be noted. First, participants were recruited in occupational health examinations, and the low lead group was also a group exposed to lead, not a strict control group. There were significant differences in some basic characteristics compared to the high lead group, which may lead to selection bias. Secondly, considering variables not included in our study, such as diet or physical activity, the consequences of these considerations on estimates of the interaction between lead exposure and polymorphism on blood pressure may be both over- and underestimations. Thirdly, due to the relatively small sample size, we did not analyze the interaction between lead and other genetic models, including co-dominant and recessive models.

In brief, we found that *CCM3* rs3804610 and *VEGFR2* rs2305948 might interact with lead to affect blood pressure, suggesting that rs3804610 and rs2305948 may serve as new targets to uncover possible personalized treatments guideline for hypertension in lead-exposed populations.

Materials and methods

Study population

This study is based on cross-sectional design, and the study population was sourced from the annual health examinations of occupational populations from 2013 to 2021 in the Pearl River Delta region of South China. After signing the consent forms, participants were asked a variety of sociodemographic questions regarding occupational health, such as age, race, sex, years of work, history of smoking, and alcohol intake. This also included a family history of CVDs and a history of taking antihypertensive drugs. Each subject was examined electrocardiogram and blood pressure by professional medical staff. Blood pressure was calculated as the average of all available systolic and diastolic readings, which were measured 3 times by a standard sphygmomanometer. Subjects having histories of cardiovascular, liver, lung, kidney or other organ diseases, psychiatric disorders, or other serious health problems and taking antihypertensive drugs were excluded. A total of 568 workers was enrolled. This study was approved by the Medical Ethics Committee of the School of Public Health at Sun Yat-sen University.

According to the current "Occupational Exposure Limits for Hazardous Factors in the Workplace" standards in China[29], the concentration of the lead smoke in most workshops did not exceed the permissible concentration-time weighted average (PC-TWA). Only one workshop(including 23 workers) showed that the highest lead smoke concentration of the short-time and long-term sampling was high than the permissible concentration. Thus, population grouping is mainly based on blood lead levels.

Collection and measurement of biological samples

Venous fasting blood (5mL EDTA anticoagulation, 5mL heparin sodium anticoagulation, 5mL non-anticoagulation) was taken from the workers in the morning for biochemical parameters analysis. We assessed the concentration of lead in whole blood in relation to participants' recent exposure to lead by using Inductively Coupled Plasma Mass Spectrometry (ICP-MS) (Agilent 7700X series, America). Blood samples were digested

via microwave-induced digestion with nitric acid. A standard solution of lead was prepared by diluting certified standard solutions (High Purity Standards, Charleston, SC, USA). Quality control materials were also used (Seronorm Trace Elements Whole Blood L-1, Trace Elements Whole Blood L-2, Norway). The lead calibration curve ranged from 0 to 1000 μg/L. Blood biochemical parameters including glucose, lipid profile and creatinine were detected by Trilogy automatic biochemical analyzer.

SNP selection and genotyping

We selected three tag SNPs: *Notch1* rs3124591, *CCM3* rs3804610 and *VEGFR2* rs2305948. *Notch1* rs3124591 is located in the 3'-UTR of chromosome 9; *CCM3* rs3804610 is located in the intron 6 of chromosome 6; *VEGFR2* rs2305948 lies within the exon region of chromosome 4. All of them are meet our inclusion criteria: (1) Minor allele frequency (MAF) > 0.1; (2) The linkage disequilibrium value of $r^2 > 0.8$; (3) Genetic balance tests (HWE) P value > 0.0001. The genotyping was done by the improved multiplex ligase detection reaction (iMLDR) method (Genesky Biotech, Shanghai) as previously described[51].

Statistical analysis

In this study, all subjects were divided into two equal groups based on the 50th percentile of blood lead levels. Categorical variables are expressed in frequency (%) and analyzed using Pearson chi-square independent test. Quantitative data obey normal distributionis expressed as the mean ± SD, and analyzed by Student's t test. The HWE tests of genotype frequencies was estimated with chi-square goodness-of-fit tests. We also conducted two independent analyses based on the presence or absence of mutated alleles to compare the difference of general characteristics. Multiple linear regression analysis was used to evaluate the association of blood pressure with lead exposure and polymorphisms. We also analyzed the interaction terms between lead and different polymorphisms on blood pressure. To address potential confounding factors that might have influenced the observed effects, we thoroughly considered several key variables in our analysis. Specifically, we identified age, years of work, sex, BMI, smoking, drinking, TC, TG, LDL-c and FBG as potential confounders based on previous research and the theoretical framework of our study, and handled by including these variables as covariates in our multiple regression models. A two-tailed p-value < 0.05 was considered significant. All statistical analysis and graphical construction were performed by Statistical Package for Social Sciences (SPSS) version 25.0 for Windows.

Data availability

Correspondence and requests for research data should be addressed to Z.Z. or Y.H.

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Declarations

Competing interests
The authors declare no competing interests.

Informed consent
Informed consent was acquired through all subjects included in this investigation.

Ethical approval
This study was approved by the Medical Ethics Committee of the School of Public Health at Sun Yat-sen University and this study was conducted in accordance with the guidelines of Medical Ethics Committees.

Additional information
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