Combined effects of nucleotide-binding domain-like receptor protein 3 polymorphisms and environmental metals exposure on chronic kidney disease

Yu-Mei Hsueh1,2, Wei-Jen Chen3, Ying-Chin Lin1,4,5, Ya-Li Huang2, Horng-Sheng Shiue6, Yuh-Feng Lin7,8, Ru-Lan Hsieh9,10 & Hsi-Hsien Chen11,12

Chronic inflammation is the cause of chronic kidney disease (CKD). The nucleotide-binding domain-like receptor protein 3 (NLRP3) inflammasome plays a vital role in the inflammation process and is associated with the regulatory effects of NLRP3 gene polymorphisms. This study evaluated the association between NLRP3 gene polymorphisms and CKD, and further explored whether the association of environmental metals with CKD varied by the NLRP3 genotypes. A total of 218 CKD patients and 427 age- and sex-matched healthy controls were recruited in this clinic-based case-control study. Patients were identified as having CKD if their estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² and stage 3–5 for at least 3 months. We examined the genotypes of fifteen common single-nucleotide polymorphisms in NLRP3 genes. Concentrations of total urinary arsenic were examined by summing of urinary inorganic arsenic species. Concentrations of selenium, cadmium, and lead were measured from blood samples. Associations between NLRP3 polymorphisms, environmental metals exposure, and CKD were evaluated using multivariable logistic regression while controlling for confounders. We observed that the odds of carrying NLRP3 rs4925650 GA/AA genotypes, NLRP3 rs1539019 CA/AA genotypes, and NLRP3 rs10157379 CT/TT genotypes were significantly higher among CKD cases compared to controls, with the adjusted odds ratio (95% confidence interval) were 1.54 (1.01–2.36), 1.56 (1.04–2.33), and 1.59 (1.05–2.38), respectively. The significant multiplicative interactions were identified between high levels of blood lead and NLRP3 rs4925650 GA/AA genotypes; high levels of blood cadmium or low levels of plasma selenium and the NLRP3 haplotype (rs4925648, rs4925650, rs12048215, and rs10754555) C-A-A-C multiplicatively interacted to increase the risk of CKD. Our results imply that NLRP3 polymorphisms may play an important role in the development of environmental metals exposure related CKD.

Chronic kidney disease (CKD) affects 8–16% of the world’s population1, resulting in CKD being a common public health problem worldwide2. Using estimated glomerular filtration rate (eGFR) < 60 mL/min/1.73 m² to

1Department of Family Medicine, Wan Fang Hospital, Taipei Medical University, Taipei, Taiwan. 2Department of Public Health, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 3Department of Medicine, Section of Epidemiology and Population Sciences, Baylor College of Medicine, Houston, TX, USA. 4Department of Family Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 5Department of Geriatric Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 6Department of Chinese Medicine, College of Medicine, Chang Gung University, Taoyuan, Taiwan. 7Graduate Institute of Clinical Medicine, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 8Division of Nephrology, Department of Internal Medicine, Shuang Ho Hospital, New Taipei, Taiwan. 9Department of Physical Medicine and Rehabilitation, Shin Kong Wu Ho-Su Memorial Hospital, Taipei, Taiwan. 10Department of Physical Medicine and Rehabilitation, School of Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 11Division of Nephrology, Department of Internal Medicine, College of Medicine, Taipei Medical University, Taipei, Taiwan. 12Division of Nephrology, Department of Internal Medicine, Taipei Medical University Hospital, Taipei, Taiwan. *email: 570713@yahoo.com.tw
define CKD, the prevalence of CKD in Taiwan was 11.9%, and only 3.5% of patients aware their disease. As the incidence of end-stage renal disease in Taiwan (426/10^5 in 2012) was the highest globally, CKD is an extremely important health issue in Taiwan.

Our previous study has reported a significantly increased odds of high total urinary arsenic levels and low plasma lycopene levels among the CKD cases compared to the health controls. We also found an increased odds of high blood cadmium and lead levels among CKD cases compared to the controls, whereas a decreased odds of high plasma selenium levels were observed among CKD cases compared to the controls. Recently, a study has shown that plasma concentrations of arsenic and lead were significantly related to the decline of renal function. In addition, a study reported that increasing concentrations of blood cadmium and lead were associated with an increased risk of proteinuria and related to decreased eGFR. These studies suggested that low levels of selenium and high levels of arsenic, cadmium, and lead may increase the risk of CKD. However, the mechanism underlying the effect of these metals on CKD remains unclear.

Inflammation is the cause of several kinds of kidney diseases, such as acute kidney injury and CKD. The nucleotide-binding domain-like receptors 3 (NLRP3) inflammasome is a multi-protein complex that plays an important role in the inflammation process. A previous study has indicated that NLRP3-induced inflammation may promote kidney inflammation and causes CKD. Also, a study has reported that the NLRP3 inflammasome may be involved in the pathogenesis of acute kidney injury, CKD, diabetic nephropathy, and crystal-related nephropathy. Recent evidence has been built to implicate arsenic, cadmium, lead, and selenium on NLRP3 related inflammatory. A study has reported that arsenic activates the NLRP3 inflammasome and induces inflammatory cell death. However, another study showed that arsenic inhibits the secretion of interleukin (IL-1β) and IL-18, which was caused by activating the NLRP3 inflammasomes in macrophages, thus, indicating that exposure to arsenic may affect inflammasome-mediated inflammation. These inconsistent findings reveal an unclear relationship between arsenic exposure and NLRP3 inflammasomes. In addition to arsenic exposure, a carp experiment showed that cadmium exposure induced apoptosis of the anterior spleen and splenic lymphocytes by activating NLRP3. Moreover, lead may activate the NLRP3 signaling pathway to cause oxidative stress and inflammation in chicken testicles, thereby reducing testicular function. In contrast, selenium has a mitigating effect by modifying activation of the NLRP3 signaling pathway in chicken testes caused by lead.

Variations in the NLRP3 gene may affect mRNA stability and NLRP3 performance. The NLRP3 gene has nine exons within its 32.9 kb sequence and is located on chromosome 1q44. There are 60 common single nucleotide polymorphisms (SNPs) that have been identified in the NLRP3 gene. Several studies have suggested that NLRP3 polymorphisms were associated with the risk of cardiovascular diseases. A study has found that the 50-year-old subjects with NLRP3 rs7512998 CC or CT genotypes had a higher blood pressure levels compared to those with TT genotype. In Chinese Han population, NLRP3 rs10754556 CC genotype was associated with the occurrence of coronary artery disease compared to those with CG or GG genotypes. The NLRP3 rs4612666 T allele is associated with an increased risk of aorta sclerosis-like ischemic stroke. To date, few studies have explored the association between NLRP3 gene polymorphisms and CKD. The present study aimed to examine the association between NLRP3 genotypes and CKD, and to further explore whether NLRP3 gene polymorphisms may modify the associations of total urinary arsenic, blood cadmium, and lead, and plasma selenium with CKD.

Materials and methods

Study subjects. This study was a clinic-based case–control study. In total, 218 clinically confirmed CKD patients and 427 age- and sex-matched controls who volunteered to participate in this study were recruited previously. All participants measured serum creatinine concentrations by isotope dilution mass spectrometry (IDMS). The eGFR (mL/min/1.73 m²) was calculated using the equation: 186.3 × (serum creatinine (mg/dL))^−1.154 × (age)^−0.203 × (0.742 for females) × 0.742 for females) × 1.23 for males). CKD patients were clinically diagnosed at the Department of Internal Medicine/Nephrology, Taipei Medical University Hospital and Taipei Municipal Wan Fang Hospital, with an eGFR < 60 mL/min/1.73 m² for at least 3 months (stages 3–5) and without hemodialysis according to KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Matched controls, with no CKD diagnosis, were recruited from receiving adult and senior citizen health examinations in the Department of Family Medicine. The Research Ethics Committee of Taipei Medical University approved this study (TMU-Joint Institutional Review Board, N201912058) which was conducted in accordance with the Declaration of Helsinki. All participants provided informed consent before the questionnaire interview and specimens collection.

Interview and specimen collection. The questionnaire interview and the urine and blood samples collection were described previously. We measured the arsenic species concentrations in single spot urine samples. EDTA-vacuum syringes were used to collect 5–8 mL peripheral blood samples, and the plasma, red blood cell, and buffy coat were separated. Buffcoast was separated for DNA extraction and to determine NLRP3 polymorphisms. We analyzed the cadmium and lead concentrations from red blood cells, and measured selenium concentrations from plasma.

Environmental metals exposure measurement. Urinary concentrations of inorganic-related arsenic species, including trivalent arsenite (As(III)), pentavalent arsenate (As(V)), monomethylarsonic acid (MMA(IV)), and dimethylarsinic acid (DMA(IV)) were measured as described previously. We assessed total urinary arsenic concentrations by summing the urinary concentrations of As(III), As(V), MMA(IV), and DMA(IV). The measure of arsenic in urine specimens was a direct method of excluding nontoxic organic arsenic that contributed to total arsenic exposure. Urinary concentrations were adjusted for urinary creatinine concentrations due to variations in the hydration state. In addition, concentrations of blood cadmium and lead and plasma selenium were determined.
as described previously. The validity and reliability of the environmental metals exposure measurement are shown in Supplementary Table S1.

Determination of the genetic polymorphisms. DNA was extracted by digestion with proteinase K followed by phenol and chloroform. We selected 15 common NLRP3 SNPs based on their minor-allele frequencies (≥ 0.2) in the Han Chinese in the Beijing HapMap database. The Agena Bioscience MassARRAY iPLEX system was used according to the manufacturer’s instructions (National Genome Medicine Center, Taipei, Taiwan) to determine the genotypes of 15 SNPs, including rs4925654, rs4925650, rs12239046, rs4925648, rs10925025, rs10925019, rs1539019, rs3806265, rs10925026, rs10157379, rs12143966, rs10754555, rs3806268, rs12048215, and rs12137901. Among them, rs12137901 did not meet the Hardy–Weinberg equilibrium and was excluded.

Statistical analysis. Differences in the continuous variables were compared between the two groups using the Wilcoxon rank-sum test. The Kruskal–Wallis test was used to compare continuous variables of more than two groups. Multiple logistic regression models were performed estimating odds ratios (ORs) and 95% confidence intervals (CIs) to evaluate the associations between NLRP3 polymorphism, environmental metal exposure, and CKD while adjusting for confounders. The measures of metal concentrations were categorized into three groups based on the tertile distribution of concentrations among controls. We further treated each tertile group as an ordinal variable in the models to conduct the trend test. Additionally, multiple linear regression models were used to assess associations between NLRP3 polymorphisms and eGFR while adjusting for confounders. Confounding was informed by prior knowledge and met the criterion that changed the ORs of exposure variables at least by 10% when adding to models assessing between environmental metal exposure and CKD. We explored the interaction by estimating the combined effects of environmental metals exposure (median of concentrations among controls as a cutoff point) and NLRP3 genotypes on CKD. The product term was added to the logistic regression models to conduct for the multiplicative interaction between metal and NLRP3 genotypes. The SAS 9.4 (SAS Institute, Cary, NC, USA) was used for statistical analyses. A two-sided p value < 0.05 was considered statistically significant.

Results

The sociodemographic characteristics, eGFR, lifestyle, and disease histories of diabetes and hypertension between CKD cases and controls are shown in Table 1. The mean and standard deviation of age and eGFR in the 218 CKD patients and 427 controls were 65.11 ± 13.52 and 64.21 ± 12.49 years, and 31.54 ± 14.57 and 84.21 ± 15.62 mL/min/1.73 m² respectively. The CKD cases had a lower level of education. Most cases were less likely to have habits of drinking coffee, tea, and alcohol than controls. The CKD patients had significantly increased odds of regularly used analgesics compared to controls, with OR (95% CI) = 2.94 (1.58–5.44). The odds of having disease histories of diabetes and hypertension were 3–5-fold increase among cases compared to controls.

Table 2 presents the association between 14 NLRP3 gene polymorphisms and CKD. We observed that the odds of carrying NLRP3 rs4925650 GA/AA genotypes were 1.54-fold (95% CI 1.15–3.34) increased among CKD cases compared to controls after adjusting for covariates. In addition, participants with CKD had a 1.56–1.79 fold increased odds of carrying NLRP3 rs12239046 CT/TT, NLRP3 rs10925025 AG/GG, NLRP3 rs1539019 CA/AA, NLRP3 rs10925026 CA/AA, and NLRP3 rs10157379 CT/TT genotypes compared to controls. After additionally adjusting environmental metals exposure, we observed that NLRP3 rs4925650 [GA/AA vs. GG, OR (95% CI) = 1.89 (0.98–3.35)], NLRP3 rs1539019 [CA/AA vs. CC, OR (95% CI) = 1.50 (0.94–2.41)] were associated with CKD though the confidence interval marginally included the null value. No association was found between other NLRP3 genotypes and CKD after adjusting for covariates and metals concentrations. NLRP3 rs4925650, NLRP3 rs1539019, and NLRP3 rs10157379 genotypes were selected for further analyzed the effect modification of gene and metal on CKD.

LD and haplotype analyses revealed that the NLRP3 genes exhibited the four haplotype blocks shown in Supplementary Figure S1. D’ of Lewontin of the haplotype NLRP3 block 1 (NLRP3 rs4925648, NLRP3 rs4925650, NLRP3 rs12048215, and NLRP3 rs10754555), NLRP3 block 2 (NLRP3 rs3806265 and NLRP3 rs38062628), NLRP3 block 3 (NLRP3 rs10925019 and NLRP3 rs4925654), and NLRP3 block 4 (NLRP3 rs1539019, NLRP3 rs10925025, NLRP3 rs12143966, NLRP3 rs12239046, NLRP3 rs10925026, and NLRP3 rs10157379) ranged from 0.99 to 1.00. The association between the NLRP3 gene haplotypes and CKD is shown in Table 3. We observed that the odds of C-G-A-C and C-G-A-G haplotypes in the NLRP3 block 1 respectively decreased by 47% and 52% among the CKD cases compared to the controls. In addition, the combined T-G-G-G, C-G-A-C, C-G-A-G, and C-G-G-G haplotypes in the NLRP3 block 1 were significantly inversely associated with CKD compared to the C-A-A-C haplotype. These associations remained statistically significant after further adjusting for metals concentrations in the models. The odds of C-A haplotype in NLRP3 block 3 were decreased by 35% among the CKD cases compared to the controls. No association was observed between NLRP3 blocks 2 and 4 and CKD.

Associations between NLRP3 rs4925650, NLRP3 rs1539019, and NLRP3 rs10157379 genotypes and eGFR are shown in Table 4. Participants who carried the NLRP3 rs4925650 AA genotype decreased 6.72 mL/min/1.73 m² of eGFR when compared to those carrying the GG genotype. Participants who carried the NLRP3 rs1539019 A allele and the NLRP3 rs10157379 T allele decreased eGFR by 6 mL/min/1.73 m² when compared with those carrying the NLRP3 rs1539019 C allele and the NLRP3 rs10157379 C allele. These results showed no changes after adjusting for blood cadmium and lead or plasma selenium levels in models.

Environmental metals exposure was found to be associated with CKD in our study (Supplementary Table S2). As the levels of total urinary arsenic and blood cadmium and lead increased, the OR of CKD increased.
significantly in a dose–response manner. In contrast, as the levels of plasma selenium increased, the OR of CKD decreased significantly in a dose–response relationship. No difference was observed in comparing concentrations of environmental metals exposure by different genotypes of \(\text{NLRP3} \) rs4925650, \(\text{NLRP3} \) rs1539019, and \(\text{NLRP3} \) rs10157379.

Figure 1 shows the combined effect of \(\text{NLRP3} \) rs4925650, \(\text{NLRP3} \) rs1539019, \(\text{NLRP3} \) rs10157379, and levels of environmental metals exposure on the CKD. The trend analysis showed that the OR of CKD increased gradually with exposure to no risk factors, one risk factor, or two risk factors (risk genotypes, high levels of arsenic, cadmium, and lead, or low levels of selenium). We observed that the odds of carrying \(\text{NLRP3} \) rs4925650 GA/AA genotypes and high levels of blood lead (> 37.40 μg/L) were 5.03-fold increased (95% CI 2.46–10.27) among CKD cases compared to controls (Fig. 1C). The p-value of the interaction term of \(\text{NLRP3} \) rs4925650 and levels of blood lead was 0.0229, which indicated a multiplicative interaction between \(\text{NLRP3} \) rs4925650 and blood lead on CKD. In addition, we observed that \(\text{NLRP3} \) block 1 (risk haplotype: C-A-A-C) interacted with total urinary arsenic, blood lead and cadmium, and plasma selenium to significantly enhance the OR of CKD, respectively (Fig. 2). High levels of blood cadmium and the \(\text{NLRP3} \) block 1 C-A-A-C haplotype, and low levels of plasma selenium and the \(\text{NLRP3} \) block 1 C-A-A-C haplotype significantly and multiplicatively interacted to increase the OR of CKD, respectively.

Discussion
To the best of our knowledge, this study is the first to evaluate the associations between \(\text{NLRP3} \) polymorphisms, environmental metals exposure, and CKD. We found that the odds of carrying \(\text{NLRP3} \) rs4925650 GA/AA genotypes, \(\text{NLRP3} \) rs1539019 CA/AA genotypes, and \(\text{NLRP3} \) rs10157379 CT/TT genotypes were significantly higher among CKD cases compared to controls. In addition, certain \(\text{NLRP3} \) genotypes were interacting

| Variables | CKD cases (N = 218) | Controls (N = 427) | Age-sex adjusted OR (95% CI) |
|-----------|---------------------|--------------------|-----------------------------|
| Sex       |                     |                    |                             |
| Male      | 133 (61.01)         | 263 (61.59)        | 1.00                        |
| Female    | 85 (38.99)          | 164 (38.41)        | 1.04 (0.74–1.46)            |
| Age       | 65.11 ± 13.52       | 64.22 ± 12.49      | 1.01 (0.99–1.02)            |
| eGFR (mL/min/1.73m²) | 31.54 ± 14.57   | 84.21 ± 15.62      | 0.35 (0.20–0.60)**          |
| Educational level |                    |                    |                             |
| Illiterate/elementary school | 90 (41.28) | 97 (22.83) | 1.00** |
| Junior/senior high school | 72 (32.03) | 150 (35.13) | 0.49 (0.33–0.74)** |
| College and above | 56 (25.69) | 180 (42.15) | 0.31 (0.20–0.48)** |
| Cigarette smoking |                    |                    |                             |
| Non-smoker | 160 (73.39)         | 311 (72.83)        | 1.00                        |
| Former smoker | 33 (15.14)         | 74 (17.33)         | 0.85 (0.53–1.39)            |
| Current smoker | 25 (11.47)         | 42 (9.84)          | 1.21 (0.69–2.12)            |
| Alcohol consumption |                    |                    |                             |
| Never | 179 (82.11) | 274 (64.17) | 1.00 |
| Occasional or frequently | 39 (17.89) | 153 (35.83) | 0.36 (0.24–0.55)** |
| Coffee consumption |                    |                    |                             |
| Never | 170 (77.98) | 218 (51.05) | 1.00 |
| Occasional or frequently | 48 (22.02) | 209 (48.95) | 0.29 (0.20–0.43)** |
| Tea consumption |                    |                    |                             |
| Never | 123 (56.42) | 149 (34.89) | 1.00 |
| Occasional or frequently | 95 (43.58) | 278 (65.11) | 0.41 (0.29–0.58)** |
| Analgesic usage |                    |                    |                             |
| No/yes as-needed basis | 192 (88.07) | 408 (95.55) | 1.00 |
| Yes, routinely | 26 (11.93) | 19 (4.45) | 2.94 (1.58–5.44)** |
| Diabetes |                    |                    |                             |
| No | 132 (60.55) | 383 (89.70) | 1.00 |
| Yes | 86 (39.45) | 44 (10.30) | 5.71 (3.77–8.66)** |
| Hypertension |                    |                    |                             |
| No | 94 (43.12) | 298 (69.79) | 1.00 |
| Yes | 124 (56.88) | 129 (30.21) | 3.14 (2.22–4.44)** |

Table 1. Sociodemographic characteristics, lifestyle and disease histories, and eGFR between the CKD cases and controls, and the ORs of these variables for CKD. Values are expressed as mean ± standard deviation or number (%) of cases and controls. CKD chronic kidney disease, eGFR estimated glomerular filtration rate, OR odds ratio, CI confidence interval. ***p < 0.001. *p < 0.05 for the trend test. §Age adjusted OR and 95% CI. a Sex adjusted OR and 95% CI.
| NLRP3 genotypes | CKD cases | Controls | Age-sex adjusted ORs (95% CI) | Multivariate adjusted ORs (95% CI)a |
|-----------------|-----------|----------|-----------------------------|----------------------------------|
| rs4925654 G>A   |           |          |                             |                                  |
| GG              | 159 (73.27) | 303 (71.13) | 1.00                        | 1.00                             |
| GA              | 54 (24.88)  | 113 (26.53) | 0.90 (0.62–1.32)            | 0.72 (0.46–1.14)                 |
| AA              | 4 (1.84)    | 10 (2.35) | 0.75 (0.23–2.43)            | 0.36 (0.08–1.51)                 |
| GA/AA versus GG | 58 (26.73)  | 123 (28.87) | 0.88 (0.62–1.25)            | 0.70 (0.45–1.08)                 |
| rs4925650 G>A   |           |          |                             |                                  |
| GG              | 59 (27.06)  | 133 (31.44) | 1.00                        | 1.00                             |
| GA              | 101 (48.52) | 210 (49.65) | 1.08 (0.73–1.59)            | 1.37 (0.87–2.16)                 |
| AA              | 58 (24.56)  | 80 (18.91) | 1.62 (1.02–2.56)*           | 1.96 (1.15–3.34)*                |
| GA/AA versus GG | 159 (72.94) | 290 (65.86) | 1.23 (0.85–1.77)            | 1.34 (1.01–2.36)*                |
| rs12239046 C>T  |           |          |                             |                                  |
| CC              | 70 (32.11)  | 166 (38.88) | 1.00                        | 1.00                             |
| CT              | 114 (52.29) | 189 (44.37) | 1.45 (1.01–2.09)*           | 1.77 (1.16–2.73)**               |
| TT              | 34 (15.60)  | 72 (16.86) | 1.12 (0.68–1.84)            | 1.10 (0.62–1.96)                 |
| CT/TT versus CC | 148 (67.89)| 261 (61.12) | 1.36 (0.96–1.92)*            | 1.56 (1.04–2.34)*                |
| rs4925648 C>T   |           |          |                             |                                  |
| CC              | 124 (56.88) | 234 (54.80) | 1.00                        | 1.00                             |
| CT              | 78 (35.78)  | 162 (35.94) | 0.92 (0.65–1.30)            | 0.94 (0.62–1.41)                 |
| TT              | 16 (7.34)   | 31 (7.26) | 0.98 (0.52–1.87)            | 0.98 (0.47–2.04)                 |
| CT/TT versus CC | 143 (67.89)| 261 (61.12) | 1.36 (0.96–1.92)*            | 1.56 (1.04–2.34)*                |
| rs10925025 G>A  |           |          |                             |                                  |
| AA              | 70 (31.94)  | 165 (38.73) | 1.00                        | 1.00                             |
| AG              | 113 (52.07) | 189 (44.37) | 1.43 (0.99–2.06)*           | 1.77 (1.15–2.72)**               |
| GG              | 34 (15.60)  | 72 (16.90) | 1.12 (0.68–1.83)            | 1.10 (0.62–1.95)                 |
| AG/GG versus AA | 147 (67.74)| 261 (61.27) | 1.34 (0.95–1.90)*            | 1.56 (1.04–2.33)*                |
| rs10925019 C>T  |           |          |                             |                                  |
| CC              | 102 (46.79) | 199 (46.60) | 1.00                        | 1.00                             |
| CT              | 101 (46.33) | 182 (42.62) | 1.09 (0.77–1.53)            | 0.98 (0.65–1.46)                 |
| TT              | 15 (6.89)   | 46 (10.77) | 0.64 (0.34–1.20)            | 0.78 (0.38–1.61)                 |
| CT/TT versus CC | 143 (67.89)| 261 (61.12) | 1.36 (0.96–1.92)*            | 1.56 (1.04–2.34)*                |
| rs1539019 C>A   |           |          |                             |                                  |
| CC              | 72 (33.03)  | 166 (39.06) | 1.00                        | 1.00                             |
| CA              | 113 (51.83) | 189 (44.37) | 1.40 (0.97–2.01)*           | 1.76 (1.15–2.72)**               |
| AA              | 34 (15.14)  | 70 (16.47) | 1.09 (0.66–1.79)            | 1.09 (0.61–1.96)                 |
| CA/AA versus CC | 146 (66.97)| 259 (60.94) | 1.31 (0.93–1.85)            | 1.56 (1.04–2.33)*                |
| rs3806265 T>C   |           |          |                             |                                  |
| TT              | 68 (31.19)  | 111 (26.06) | 1.00                        | 1.00                             |
| TC              | 108 (49.54) | 215 (50.47) | 0.79 (0.51–1.24)            | 0.79 (0.51–1.24)                 |
| CC              | 42 (19.27)  | 100 (23.47) | 0.70 (0.41–1.19)            | 0.70 (0.41–1.19)                 |
| CT/CC versus TT | 150 (68.81)| 315 (73.94) | 0.76 (0.50–1.16)            | 0.76 (0.50–1.16)                 |
| rs10925026 A>C  |           |          |                             |                                  |
| CC              | 70 (32.11)  | 166 (39.06) | 1.00                        | 1.00                             |
| CA              | 113 (52.29) | 187 (44.00) | 0.83 (0.56–1.21)            | 1.79 (1.16–2.74)**               |
| AA              | 34 (15.60)  | 72 (16.94) | 0.69 (0.43–1.11)            | 1.10 (0.62–1.95)                 |
| CA/AA versus CC | 148 (68.35)| 259 (60.94) | 0.79 (0.55–1.13)            | 1.57 (1.05–2.35)*                |
| rs10157379 T>C  |           |          |                             |                                  |
| CC              | 69 (31.65)  | 164 (38.68) | 1.00                        | 1.00                             |
| CT              | 114 (52.29) | 189 (44.37) | 1.45 (1.01–2.07)*           | 1.79 (1.16–2.75)**               |
| TT              | 35 (16.06)  | 71 (16.75) | 1.18 (0.72–1.93)            | 1.15 (0.65–2.04)                 |
| CT/TT versus CC | 149 (68.35)| 259 (60.94) | 1.38 (0.97–1.95)*            | 1.59 (1.05–2.38)*                |
| rs12143966 A>G  |           |          |                             |                                  |
| GG              | 57 (26.15)  | 125 (29.83) | 1.00                        | 1.00                             |
| GA              | 112 (51.38) | 191 (45.58) | 1.29 (0.87–1.95)            | 1.41 (0.89–2.21)                 |
| AA              | 49 (22.48)  | 103 (24.58) | 1.04 (0.66–1.66)            | 1.14 (0.67–1.96)                 |
| GA/AA versus GG | 161 (73.85)| 294 (70.17) | 1.20 (0.83–1.74)            | 1.31 (0.86–2.01)                 |
| rs10754555 C>G  |           |          |                             |                                  |
| Continued       |           |          |                             |                                  |
coagulation network identified as a consensus binding site for epidermal growth factor that may influence the vertebrate blood with primary gouty arthritis in the Chinese Han population. The innate immune system is the most characteristic inflammasome is NLRP3, which responds to endogenous C-type lectin-like receptors, toll-like receptors, and retinoic acid-inducible gene I-like receptors act as sensors of unknown. The pattern recognition receptors, such as nucleotide oligomerization domain-like receptors (NLRs), play a key role in the underlying inflammatory response in many chronic diseases including CKD.

Genetic variation in the NLRP3 gene may be an important determinant of the degree of the immune inflammatory response, which affects susceptibility to inflammatory diseases. In the present study, NLRP3 rs1539019 CA/AA genotypes significantly increased with the risk of CKD after adjusting for confounders. Various studies have evaluated the association between NLRP3 gene polymorphisms and CKD. The association between NLRP3 rs10157379 CT/TT genotypes on CKD risk was significant. However, another study found no significant association between the NLRP3 rs1539019 polymorphism and several health-related outcomes. Patients with chronic hepatitis C virus and the NLRP3 rs1539019 AA genotype do not respond to interferon therapy, but the NLRP3 rs1539019 TT genotype is related to pneumonia in Chinese coal workers. In addition, it has been reported that the NLRP3 rs1539019 A allele is related to circulating fibrinogen concentration and therefore to the risk of cardiovascular disease. The rs1539019 G>T locus is close to a 12-nucleotide sequence identified as a consensus binding site for epidermal growth factor that may influence the vertebrate blood coagulation network. NLRP3 rs1539019 A allele has been indicated to increase circulating fibrinogen levels, an indicator of inflammation, which may be associated with blood coagulation, followed by leading to CKD. However, another study found no significant association between the NLRP3 rs1539019 polymorphism and the risk of essential hypertension in a Japanese population. The NLRP3 rs1539019 polymorphism was not associated with primary gouty arthritis in the Chinese Han population. NLRP3 rs1539019 is an intronic polymorphism and it is unclear how genetic variations in introns affect gene function. However, one study reported that many transcription factors bind to intron sites, and these intron sites may play a role regulating gene expression.

In addition to NLRP3 rs1539019 polymorphism, we also found that NLRP3 rs4925650 GA/AA genotypes and NLRP3 rs10157379 CT/TT genotypes significantly increased the risk of CKD after adjusting for confounders. Hence, the effect of NLRP3 rs4925650 GA/AA genotypes and NLRP3 rs10157379 CT/TT genotypes on susceptibility to CKD appears to be independent of age, sex, educational level, consumption of tea, alcohol, coffee, analgesic usage, and disease histories of diabetes and hypertension.

| NLRP3 genotypes | CKD cases | Controls | Age-sex adjusted ORs (95% CI) | Multivariate adjusted ORs (95% CI) |
|-----------------|-----------|----------|-----------------------------|----------------------------------|
| CC              | 85 (38.99)| 154 (36.15) | 1.00                        | 1.00                             |
| CG              | 102 (46.79)| 199 (46.71) | 0.93 (0.65–1.35)           | 0.87 (0.57–1.32)                 |
| GG              | 31 (14.22)| 73 (17.14)  | 0.78 (0.47–1.28)           | 0.69 (0.39–1.23)                 |
| CG/GG versus CC| 133 (61.01)| 271 (63.85) | 0.89 (0.64–1.15)           | 0.82 (0.55–1.21)                 |

rs3806268 A>G

| Genotypes | CKD cases | Controls | Age-sex adjusted ORs (95% CI) | Multivariate adjusted ORs (95% CI) |
|-----------|-----------|----------|-----------------------------|----------------------------------|
| AA        | 68 (31.19)| 113 (26.59)| 1.00                        | 1.00                             |
| AG        | 108 (49.54)| 212 (49.88) | 0.85 (0.58–1.25)           | 0.84 (0.54–1.31)                 |
| GG        | 42 (19.27)| 100 (23.53) | 0.71 (0.44–1.13)           | 0.73 (0.43–1.26)                 |
| AG/GG versus AA | 150 (68.81)| 312 (73.41) | 0.81 (0.56–1.15)           | 0.80 (0.53–1.22)                 |

rs12048215 A>G

| Genotypes | CKD cases | Controls | Age-sex adjusted ORs (95% CI) | Multivariate adjusted ORs (95% CI) |
|-----------|-----------|----------|-----------------------------|----------------------------------|
| AA        | 103 (47.25)| 192 (44.96) | 1.00                        | 1.00                             |
| AG        | 93 (42.66)| 185 (43.33) | 0.94 (0.67–1.33)           | 0.93 (0.62–1.41)                 |
| GG        | 22 (10.09)| 50 (11.71)  | 0.83 (0.47–1.44)           | 0.95 (0.50–1.77)                 |
| AG/GG versus AA | 115 (52.75)| 235 (55.02) | 0.92 (0.66–1.28)           | 0.94 (0.64–1.38)                 |

Table 2. The association between NLRP3 gene polymorphisms and CKD. NLRP3 rs3806265 and rs10754555 were missing for one participant. NLRP3 rs4925654, rs10925025, rs1539019, rs10925026, and rs3806268 were missing for two participants. NLRP3 rs10157379 was missing for three participants. NLRP3 rs4925650 was missing for four participants. NLRP3 rs12143966 was missing for nine participants. CKD chronic kidney disease, NLRP3 nucleotide-binding domain-like receptors 3, OR odds ratio, CI confidence interval. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.005 for the trend test. a Adjusted for age, sex, educational level, alcohol, coffee and tea consumption, analgesic usage, and disease histories of diabetes and hypertension.
As diabetes is a recognized risk factor for CKD, 

**Table 3.** The association between NLRP3 gene haplotypes and CKD. CKD, chronic kidney disease; NLRP3, nucleotide-binding domain-like receptors 3; OR, odds ratio; CI, confidence interval. *0.05 ≤ \( p \) < 0.1, * \( p \) < 0.05.

| NLRP3 block 1: rs4925648, rs4925650, rs12048215, and rs10754555 | CKD Cases | Controls | Age-sex adjusted ORs (95% CI) | Multivariate adjusted ORs (95% CI)* |
|---|---|---|---|---|
| C-A-A-C | 215 (49.65) | 370 (43.94) | 1.00 | 1.00 |
| T-G-G-G | 108 (24.94) | 224 (26.60) | 0.84 (0.63–1.11) | 0.79 (0.57–1.10) |
| C-G-A-C | 56 (12.93) | 133 (15.80) | 0.73 (0.51–1.04)* | 0.63 (0.42–0.95)* |
| C-G-A-G | 27 (6.24) | 62 (7.36) | 0.75 (0.46–1.22) | 0.48 (0.27–0.85)* |
| C-G-G-G | 27 (6.24) | 53 (6.29) | 0.79 (0.49–1.28) | 0.82 (0.47–1.43) |
| T-G-G-G/C-G-A-C/G-C-A-G/C-G-G-G versus C-A-A-C | 218 (50.35) | 472 (54.06) | 0.79 (0.63–0.99)* | 0.70 (0.54–0.92)* |

**Table 4.** The association between NLRP3 gene polymorphisms and eGFR. eGFR, estimated glomerular filtration rate (mL/min/1.73 m²); \( \beta \), Regression coefficient; SE, Standard error of regression coefficient. *Adjusted for age, sex, educational level, alcohol, coffee and tea consumption, analgesic usage, disease histories of diabetes and hypertension.

| NLRP3 genotypes | Genotypes/Alleles | \( \beta \) (SE)a | \( p \) values |
|---|---|---|---|
| rs4925650 G>A | GA versus GG | –1.32 (2.40) | 0.582 |
| | AA versus GG | –7.62 (2.90) | 0.009 |
| | GA/AA versus GG | –3.30 (2.26) | 0.145 |
| | A versus G | –3.06 (2.26) | 0.175 |

**Table 3.** The association between NLRP3 gene haplotypes and CKD. CKD, chronic kidney disease, NLRP3, nucleotide-binding domain-like receptors 3, OR, odds ratio, CI, confidence interval. \( \text{A} \leq 0.05 \leq \beta < 0.1, * \beta < 0.05. \)

*a Adjusted for age, sex, educational level, alcohol, coffee and tea consumption, analgesic usage, and disease histories of diabetes and hypertension.

the activation of the NLRP3 inflammasome and subsequent secretion of interleukin 1β, which was involved in the pathogenesis of diabetes*. As diabetes is a recognized risk factor for CKD, NLRP3 genes may be involved in diabetes-related CKD. Additionally, the haplotype analyses were performed showing that the NLRP3 block
C-G-A-C, C-G-A-G, C-G-G-G, or T-G-G-G haplotypes significantly decreased the OR of CKD compared to that of the C-A-A-C haplotype (which includes the \textit{NLRP3} rs4925650 A allele). To date, epidemiological studies evaluating the effect of \textit{NLRP3} polymorphisms on CKD are limited, further studies are needed to explore the possible mechanism of the associations between these genotypes, the haplotype, and CKD.

Several in vitro and in vivo studies have indicated that metals exposure may affect \textit{NLRP3} functional changes. An in vitro study has reported that the insulin resistance induced by NaAsO$_2$ is due to activation of the \textit{NLRP3} inflammasome\cite{47}. Chicken experiments have shown that the \textit{NLRP3} signaling pathway is activated by lead-induced oxidative stress after lead administration, which causes testicular damage\cite{16}. Other animal studies have shown that cadmium chloride induces testicular injury\cite{48} or liver injury\cite{49} in mice by activating the \textit{NLRP3} signaling pathway. A selenium-rich basal diet may inhibit lipopolysaccharide-induced inflammation in chicken liver by suppressing the toll like receptor 4-nuclear factor kB-NLRP3 signaling pathway\cite{50}. Also, a study has reported that dietary selenium attenuates \textit{Staphylococcus aureus} mastitis in mice by inhibiting the NLRP3 inflammasome\cite{51}.

Our study found no difference when comparing concentrations of environmental metals exposure by different genotypes of \textit{NLRP3} rs4925650, \textit{NLRP3} rs1539019, and \textit{NLRP3} rs10157379, which suggests that metals exposure and the \textit{NLRP3} genes have independent effects on CKD. Additional studies are needed to better understand the effect of environmental metals exposure in \textit{NLRP3} function and its mechanism.

In the present study, we observed that high levels of blood lead and \textit{NLRP3} rs4925650 GA/AA genotypes significantly interacted to increase the risk of CKD after multivariate adjustment. This may be because lead in the blood can induce alterations of inflammatory marker \textit{NLRP3} inflammasome activation\cite{16}, and reduced eGFR\cite{6}, leading to an increase in the risk of CKD. We also found that high levels of blood cadmium and the \textit{NLRP3} block 1 haplotype C-A-A-C multiplicatively interacted to increase the risk of CKD after adjusting for multiple risk factors. Evidence has shown that cadmium in the blood may inhibit heme oxygenase 1 and nuclear factor erythroid 2-related factor 2, and activate the \textit{NLRP3} inflammasome\cite{48} or increase reactive oxygen species to activate the \textit{NLRP3} inflammasome\cite{49}, and decreased eGFR\cite{6}, which may jointly cause CKD pathogenesis\cite{52}. In addition, we observed that low plasma selenium level and the \textit{NLRP3} block 1 haplotype C-A-A-C multiplicatively interacted to increase the risk of CKD after adjusting for multiple risk factors. Studies have found that a low level of plasma selenium may not inhibit the expression of \textit{NLRP3}\cite{51}, or downregulate the toll-like receptor 4-nuclear factor-kb-\textit{NLRP3} signaling pathway\cite{50}, which may increase kidney inflammation to increase the risk of CKD\cite{53}. Our study did not precisely measure the levels of serum \textit{NLRP3} inflammasome. Therefore, whether the \textit{NLRP3}
inflammasome was affected by levels of blood cadmium and lead, low levels of plasma selenium or the NLRP3 polymorphisms remain unknown. The underlying mechanism of joint effect of environmental metals exposure and NLRP3 polymorphisms affecting CKD needs further exploration. The sample size of this study was small with limited statistical power. Further studies with larger sample size are needed to improve the precision of point estimates when assessing the effect modification of NLRP3 gene polymorphisms and environmental metals exposure affecting CKD needs further exploration. The estimates of OR were adjusted for age, sex, educational level, alcohol, coffee and tea consumption, analgesic usage, disease histories of diabetes and hypertension, and levels of other metals.

**Conclusions**
In conclusion, the present study found evidence that the NLRP3 rs4925650 GA/AA genotypes or NLRP3 block 1 haplotype C-A-A-C altered the risk of CKD related to high levels of blood lead and cadmium, or low levels of plasma selenium. Future studies are warranted to measure the levels of serum NLRP3 inflammasome, to elucidate the biological mechanism underlying the associations between NLRP3 polymorphisms, environmental metals exposure, and CKD.

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Author contributions
Y.L.H., H.S.S., and R.L.H. partly contributed to the conception and design of the work, and H.H.C., Y.F.L., and Y.C.L. recruited the study subjects; W.J.C. has done the experiment; Y.M.H. contributed to the statistical analysis and analyzed the data. Y.M.H. wrote the manuscript; W.J.C. reviewed and editing the manuscript; H.H.C. performed the study design and executed the whole research plan.

Competing interests
The authors declare no competing interests.

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Correspondence and requests for materials should be addressed to H.-H.C.

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