KLHL3 single-nucleotide polymorphism is associated with essential hypertension in Chinese Han population

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Abstract
Hypertension, including secondary and essential hypertension (EH) variants, is a multifactorial disease, affecting more than one billion people worldwide. Secondary hypertension results from mutations in the putative gene KLHL3 (Kelch-like protein 3); however, it has not been reported whether the KLHL3 gene polymorphisms are associated with EH. Here, we investigated the association between KLHL3 (rs2301708 and rs7444370) polymorphisms and EH in the Chinese Han population.

This case–control study included 522 subjects—260 patients with EH and 262 normotensive controls matched for age, gender, body mass index (BMI), hemoglobin A1c (HbA1c), total cholesterol (TC), triglyceride (TG), and levels of Na⁺, K⁺, and Cl⁻. The distribution of functional rs2301708 and rs7444370 polymorphisms within the KLHL3 gene was assessed through polymerase chain reaction (PCR) and restriction-fragment length polymorphism (RFLP).

There was no significant difference in allelic and genotypic frequencies of KLHL3 rs2301708 between the EH and normotensive groups; however, the rs7444370 T allele and CT genotype in females was significantly associated with a protective effect against EH (P = .001, P = .002; P = .019, P = .052), and the haplotype CT of rs2301708 and rs7444370 among females in the EH group was less than in the normotensive group (P = .000; P = .007).

The KLHL3 rs7444370 variant could be a protective factor in the pathogenesis of females’ EH.

Abbreviations: ANOVA = analysis of variance, BMI = body mass index, BP = blood pressure, BTB = bric-a-brac tramtrack broad complex, CIs = confidence intervals, CRL = cullin-RING E3 ubiquitin ligase, CUL3 = cullin3, CYP = cytochrome P450, EH = essential hypertension, Eleetion = Evaluation, and treatment of high blood pressure, GWAS = genome-wide association studies, HbA1c = hemoglobin A1c, HWE = Hardy–Weinberg equilibrium, JNC7 = Seventh Report of the Joint National Committee on Prevention, JNC8 = 2014 evidence-based guideline for the management of high blood pressure in adults: report from the panel members appointed to the Eighth Joint National Committee, KLHL3 = Kelch-like3, NCC = Na⁺/Cl⁻ cotransporter, OR = odds ratio, PCR = polymerase chain reaction, PHAII = pseudohypoaldosteronism type II, RFLP = restriction-fragment length polymorphism, ROMK = renal outer medullary potassium channel, SNPs = single-nucleotide polymorphisms, TC = total cholesterol, TG = triglyceride, WNK = with-no-lysine kinase.

Keywords: Chinese Han population, essential hypertension, haplotype, KLHL3, polymorphisms

1. Introduction
Hypertension is a devastating chronic disease that affects more than 20% of the global population.[1] The prevalence of hypertension in China was 25.2% in 2012, which was higher than that in 2002 (16.1%). Hypertension ranks tenth among the leading causes of death in China, and can cause cardiac infarction, ischemic/hemorrhagic stroke, chronic congestive heart failure, and end-stage renal disease.[2,3] Therefore, it is clinically important and urgent to seek effective treatments for hypertension. It is estimated that 90% to 95% of patients with hypertension were diagnosed with essential hypertension (EH)—a multifactorial disease caused by genetic factors, lifestyle factors, environmental factors, and overnutrition.[4,5] The etiopathogenesis of EH include a complex interaction between genetic and other factors.[6] Multiple genes or their variants related to EH are being elucidated by genome-linkage studies or genome-wide association studies (GWAS).[7] Some genetic variants have been shown to contribute to EH, according to genome-wide association studies, and are associated with secondary hypertension.[8] Researchers at our laboratory have previously shown that some CUL3 variants, associated with hypertension, may be associated with EH.[10] Single-nucleotide polymorphism (SNP) is a common pattern of variations in the human DNA sequence. SNPs occur due to...
“missense change” in amino acids during protein synthesis or in regions outside the coding region of genes as well as through changes in the regulation of genes related to gene function. SNPs underlie the different susceptibilities of humans to various forms of disease. To date, GWAS have identified several SNPs of genes, including *TPRC6*, *CYP4F2*, *ATP1B1*, *CYP2J2*, *CYP17A1*, *CD36*, and *CYP4A11*, that are very closely associated with the risk of EH progression.

In addition to these genes, there are potentially other genes that may affect the progression of EH. Kelch-like protein 3 (KLHL3), a substrate adaptor of cullin3 (CUL3), has a strong association with CUL3 in regard to protein structure and with-no-lysine kinase (WNK) isoforms; it regulates sodium, potassium, and pH as well as overall homeostasis, thus playing an important role in blood pressure regulation by increasing the activity of renal electrolyte cotransporters.\(^{18,19}\) The Cullin-RING E3 ubiquitin ligase (CRL) controls the ubiquitination of WNK and enhances levels of WNK isoforms by utilizing CUL3 and KLHL3. By stabilizing WNK isoforms, loss-of-function mutations in KLHL3 can cause pseudohypoaldosteronism type II (PHAII)—a rare Mendelian syndrome featuring hypertension.\(^{20}\) Correlations between the KLHL3 mutations and PHAII have been identified, and are speculated to play a predominant role in the renal system on the basis of observed associations of KLHL3 mutations with high blood pressure.\(^{19}\)

The relationship between variants of WNK1 or WNK4 and EH has been studied in the Chinese population.\(^{21–23}\) Some studies have identified KLHL3 rs2301708 to be associated with susceptibility to congenital sensorineural hearing loss,\(^{24}\) but only very few studies evaluated the association of KLHL3 SNPs with EH.\(^{25}\)

After studying the relationship between CUL3 variants and EH, we selected the rs2301708 and rs7444370 SNPs of KLHL3 for a haplotype analysis and literature review in the Asian and Chinese Han population. Furthermore, we aimed to examine the possible relationship between these SNPs and EH. The Han population is the largest number of ethnic groups in China and, thus far, there is no report of a relationship between KLHL3 SNPs and EH in the Han population. This study was undertaken to glean novel insights into the etiopathogenesis of EH by investigating EH-associated KLHL3 SNPs.

### 2. Methods

#### 2.1. Selection of SNPs

The analysis of haplotype block and linkage disequilibrium (LD) was conducted with the Haploview software using genotype data of the Han population from Guangzhou General Hospital of Guangzhou Military Command. In total, 265 subjects with EH and 269 normotensive control subjects were recruited. Five subjects with EH and seven normotensive control subjects with missing test data were excluded. The remaining subjects who were matched for gender, age, body mass index (BMI), biochemical markers associated with hypertension (TG, TC, HbA1c, K+, Na+, and Cl– in blood) were included in this study (Table 1). All patients with a history of EH, ranging from 3 months to 47 years, had normal-range blood pressure as they were on antihypertensive medication. As against the personal history of subjects, there was a very significant improvement in habits of smoking (≥5 cigarettes each day\(^{26}\)), alcohol consumption (≥140 g per week\(^{27}\)), and physical activity (>30 min of aerobic exercise ≥2 times per week\(^{28}\)) after post-diagnosis professional education in the EH group. All subjects in this study were of Han ethnicity. EH was diagnosed according to JNC7\(^{29}\) or JNC8\(^{30}\) guidelines: diastolic blood pressure (DBP) higher than 90 mmHg, and systolic blood pressure (SBP) higher than 140 mmHg without any antihypertensive treatments or a confirmed EH diagnosis by a specialized physician. Patients with secondary hypertension caused by other disorders or acute-phase disorders of the cardiovascular, hepatic, renal, and pulmonary systems, and other somatic diseases or with malignant tumors were excluded. Normotensive subjects were selected according to the following criteria: DBP less than 85 mmHg and SBP < 129 mmHg without any antihypertensive medications. Subjects with a current diagnosis of malignant tumors, diabetes, or acute-phase disorders of the cardiovascular, hepatic, renal, and pulmonary systems, and other somatic diseases were excluded from the normotensive control group.

#### 2.2. Scientific study ethics

The study was conducted critically in accordance with the strategies and recommendations of the guidelines and standards stipulated by the Medical Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command. The protocol (no. GZZY-JS-2012–0019) for this study was approved by the Ethics Committee of Guangzhou General Hospital of Guangzhou Military Command. All subjects provided written informed consent for study participation in accordance with the principles of the Declaration of Helsinki.

### 2.3. Subjects

Subjects were enrolled from 2012 to 2015 in the Guangzhou General Hospital of Guangzhou Military Command. In total, 265 subjects with EH and 269 normotensive control subjects were recruited. Five subjects with EH and seven normotensive control subjects with missing test data were excluded. The remaining subjects who were matched for gender, age, body mass index (BMI), biochemical markers associated with hypertension (TG, TC, HbA1c, K+, Na+, and Cl– in blood) were included in this study (Table 1). All patients with a history of EH, ranging from 3 months to 47 years, had normal-range blood pressure as they were on antihypertensive medication. As against the personal history of subjects, there was a very significant improvement in habits of smoking (≥5 cigarettes each day\(^{26}\)), alcohol consumption (≥140 g per week\(^{27}\)), and physical activity (>30 min of aerobic exercise ≥2 times per week\(^{28}\)) after post-diagnosis professional education in the EH group. All subjects in this study were of Han ethnicity. EH was diagnosed according to JNC7\(^{29}\) or JNC8\(^{30}\) guidelines: diastolic blood pressure (DBP) higher than 90 mmHg, and systolic blood pressure (SBP) higher than 140 mmHg without any antihypertensive treatments or a confirmed EH diagnosis by a specialized physician. Patients with secondary hypertension caused by other disorders or acute-phase disorders of the cardiovascular, hepatic, renal, and pulmonary systems, and other somatic diseases or with malignant tumors were excluded. Normotensive subjects were selected according to the following criteria: DBP less than 85 mmHg and SBP < 129 mmHg without any antihypertensive medications. Subjects with a current diagnosis of malignant tumors, diabetes, or acute-phase disorders of the cardiovascular, hepatic, renal, and pulmonary systems, and other somatic diseases were excluded from the normotensive control group.

**Table 1** Baseline parameters of study population.

| Parameters                        | EH group (n = 260) | Normotensive group (n = 262) | P-Value |
|----------------------------------|-------------------|-----------------------------|---------|
| Age, year                        | 77.77 ± 7.654     | 76.81 ± 9.088               | .192†   |
| Age range, year                  | 58–88             | 56–98                       | 1.000   |
| Gender, male, %                  | 50.4%             | 50.8%                       | 1.000   |
| Smoking                          | 17.31%            | 12.98%                      | .277    |
| Alcohol-consuming population     |                   |                             |         |
| Physical activity                | 80.38%            | 84.35%                      | .745    |
| BMI                              | 23.66 ± 3.866     | 23.30 ± 3.747               | .279†   |
| TG, mmol/L                       | 1.438 ± 0.024     | 1.343 ± 1.140               | .296‡   |
| TC, mmol/L                       | 4.451 ± 0.893     | 4.328 ± 0.835               | .105‡   |
| HbA1c, %                         | 5.336 ± 0.098     | 5.257 ± 0.613               | .244‡   |
| K+, mmol/L                       | 4.428 ± 0.453     | 4.381 ± 0.420               | .220‡   |
| Na+, mmol/L                      | 141.072 ± 2.77    | 140.768 ± 2.641             | .200‡   |
| Cl–, mmol/L                      | 103.127 ± 3.256   | 102.784 ± 3.357             | .235‡   |

Parameter: smoking ≥5 cigarettes each day, alcohol consumption ≥140 g per week, physical activity ≥30 min of aerobic exercise ≥2 times per week.

BMI, TC, TG, HbA1c, K+, Na+, and Cl– of recruited subjects were detected before genotyping of DNA. \(\ast P<0.05\). Chi-squared tests with Fisher exact test. \(\dagger\) Analyzed by F-test with two tails.
2.4. Collection of blood and DNA extraction

After overnight fasting, 2 to 3 mL non-anticoagulant venous blood samples were collected from subjects for laboratory tests before breakfast. These samples were then transferred within 1 to 2 h via a freezer box maintained at 2 to 10°C, to an ultra-low-temperature refrigerator (−80°C).

From these samples, genomic DNA was extracted from circulating leukocytes using the Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). The nuclei of leukocytes and the red blood cells were lysed. Afterwards, all proteins were precipitated, and this was followed by the precipitation of total DNA using a pure isopropanol solution. The DNA pellets were washed with a pure ethanol solution. Finally, total DNA pellets were rehydrated with the DNA rehydration solution and preserved in liquid nitrogen (<−190°C).

2.5. Genotyping of SNPs

Genomic DNA was isolated from circulating leukocytes in the peripheral blood in accordance with the standard operating procedure by total DNA isolation kits (Tiangen, Beijing, China). Genotyping of KLHL3 SNPs rs2301708 and rs7444370 was carried out by matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) with the Sequenom MassARRAY system (Sequenom Laboratories Inc., CA, USA). Primers used for genotyping were designed by Oligo software (v7.37): rs2301708 forward primer, 5'-ACCCCGAGGTTCTCCCATACCT-3'; rs2301708 reverse primer, 5'-CCAGTATCCCTGGCACCACACT-3'; rs7444370 forward primer, 5'-CCCTGGAATAGGGGGAAGCAGC-3', and reverse primer 5'-AGTGGTTGCCAGGACAGGACGT-3'. PCR was carried out in a 20-µL reaction mixture comprising 0.3 mM dNTP, 3.0 mM Mg2+, 1 U HotStarTaq polymerase, 1× HotStarTaq buffer (Qiagen Inc., MD, USA) and 1 µL pure DNA sample. Amplification was executed as follows: initial denaturation at 95°C for 2 min, then 11 cycles of denaturation at 94°C for 20 s, annealing at 59.5°C for 40 s, and extension at 72°C for 90 s, 24 cycles of denaturation at 94°C for 20 s, annealing at 59°C for 30 s, and extension at 72°C for 90 s followed by a final extension at 72°C for 120 s. PCR products were stored at 4°C. Restriction DNA fragments of PCR were electrophoresed on 1% agarose gels and stained with ethidium bromide. All allelic discrimination of KLHL3 was measured by the ABI3730XL (Applied Biosystems, CA, USA) using the software of GeneMapper 4.1 (95% confidence intervals [CIs]).

2.6. Statistical analysis

All statistical analysis was carried out in the SPSS 18.0 (SPSS Inc., IL, USA). Subjects with partially missing data were excluded from the statistical analysis. The haplotyping of rs2301708 and rs7444370 was done using the PHASE 2.0 (University of Manchester, Manchester, UK). Chi-squared tests with the Fisher exact test were used to analyze whether the sex differences and the genotypic distributions differed from the expected Hardy–Weinberg equilibrium (HWE) as well as to evaluate the distribution of the rs2301708 and rs7444370 genotypes and alleles between the EH and normotensive control groups. The Student’s t-test was used to compare the values of BMI and TG, TC, HbA1c, K+, Na+, and Cl− from venous blood between the EH and normotensive control groups. The odds ratio (OR) and corresponding 95% CIs were evaluated to compare the distribution of genotypes and alleles in the EH and normotensive control groups. Two-tailed estimation of significance was used for all analysis. *P < .05 was considered to be statistically significant.

3. Results

3.1. Baseline parameters of the study population

This study population comprised Han individuals—260 EH subjects (131 men and 129 women; mean ± SD [range] age 77.77 ± 7.654 [55–88] years) and 262 controls (133 men and 129 women; mean ± SD [range] 76.81±9.088 [56–98] years). The between-group age and gender ratio differences were not statistically significant (P = .193 and P = 1.000, respectively). There were no obvious differences (P > .05) between the EH and normotensive groups in lifestyle factors such as smoking (≥5 cigarettes each day), alcohol consumption (≥140 g per week), and physical activity (>30 min of aerobic exercise ≥2 times per week). Clinical vital signs and test parameters, including age, gender, weight, TG, TC, HbA1c, and serum electrolytes, were recorded. There was no statistically significant between-group difference in terms of BMI, TG, TC, HbA1c, Na+, K+, and Cl− (P-values were .280, .295,.104, .306,.215,.199, and .237, respectively; Table 1). SBP and DBP were recorded but not analyzed, because all EH patients were on antihypertensive medications for durations ranging from 3 months to 47 years; their blood pressure would be influenced by the antihypertensive medications and could not be accurately obtained. Thus, the blood pressure of the EH group was in the normal range as was that of the normotensive group.

3.2. Allele and genotype status of KLHL3 rs2301708 and rs7444370 SNPs in the EH and control groups

The frequencies of the allele and genotype status of the rs2301708 and rs7444370 SNPs are shown in Tables 2 and 3, respectively. The genotype distribution in the EH and control groups did not deviate from the HWE (P > .05). The results from the chi-squared test showed there was no significant (P > .05) between-group difference in allele and genotype frequencies of KLHL3 rs2301708 in the EH and normotensive groups (Table 2); however, rs7444370 T allele carriers among the female study population had significantly protective effects for EH (P = .001, OR = 5.336, 95% CIs = 1.798–15.839; P = .019, OR = 1.922, 95% CIs = 1.128–3.274; Table 3). Similarly, a statistically significant difference was observed between the rs7444370 CT and CC genotypes in females in the EH group and the normotensive control groups (P = .002, OR = 5.114, 95% CIs = 1.680–15.568; Table 3). After further stratification, the haplotype frequency distribution of the two SNPs of KLHL3 was statistically significant between the haplotype CT of rs7444370 and rs2301708 in females in the EH and normotensive control groups (χ2 = 13.626, P = .000, OR = 0.001, 95% CIs = 0.023–0.437; χ2 = 7.350, P = .007, OR = 0.469, 95% CIs = 0.268–0.819; Table 4).

Overall, these results suggest that the rs7444370 T allele and CT genotype, and haplotype CT of rs7444370 and rs2301708 in the female study population had a significantly protective effect on EH. The T allele and haplotype CT in all subjects had a significantly protective effect against EH, and the CT genotype showed some protective trend in all of the representatives (P = .052).
Table 2
Distribution frequency of KLHL3 rs2301708 polymorphism in EH and normotensive groups.

| Gender | Gene classification | rs2301708 | EH group | Normotensive group | P   | OR (95% CI) |
|--------|---------------------|-----------|----------|--------------------|-----|-------------|
|        | Allele              | C         | 288      | 289                | .95 | 1.009 (0.791–1.288) |
|        | T                   | 232       | 235      |                    |     |              |
|        | **Genotype**        |           |          |                    |     |              |
|        | CC                  | 81        | 83       | 1                  |     |              |
|        | CT                  | 126       | 123      | .841               | 0.963 (0.642–1.413)  |
|        | TT                  | 53        | 56       | 1                  | 1.031 (0.635–1.674)  |
| Male   | Allele              | C         | 159      | 151                | .378| 1.176 (0.831–1.663)  |
|        | T                   | 103       | 115      |                    |     |              |
| Female | Allele              | C         | 129      | 138                | .481| 0.870 (0.562–1.346)  |
|        | T                   | 129       | 120      |                    |     |              |

*P < .05.

Table 3
Distribution frequency of KLHL3 rs7444370 polymorphism in EH and normotensive groups.

| Gender | Gene classification | rs7444370 | EH group | Normotensive group | P   | OR (95% CI) |
|--------|---------------------|-----------|----------|--------------------|-----|-------------|
|        | Allele              | C         | 408      | 483                | .019*| 1.922 (1.128–3.274)  |
|        | T                   | 22        | 41       |                    |     |              |
|        | **Genotype**        |           |          |                    |     |              |
|        | CC                  | 238       | 223      | 1                  |     |              |
|        | CT                  | 22        | 37       | .052               | 1.796 (1.027–3.137)  |
|        | TT                  | 0         | 2        | 1.076 (0.589–1.965) |
| Male   | Allele              | C         | 244      | 245                | .74 | 1.162 (0.604–2.235)  |
|        | T                   | 18        | 21       |                    |     |              |
| Female | Allele              | C         | 254      | 238                | .001*| 5.336 (1.798–15.839)  |
|        | T                   | 4         | 20       |                    |     |              |

*P < .05.

Table 4
Haplotype frequency distributions of KLHL3 2 SNPs in EH and normotensive groups.

| KLHL3  | Haplotype | EH group (ratios) | Normotensive group (ratios) | $\chi^2$ | P   | OR (95% CI) |
|--------|-----------|------------------|-----------------------------|---------|-----|-------------|
|        | CC        | 268:260          | 247:262                     | 0.516   | .495| 1.093 (0.857–1.395)  |
|        | CT        | 20:260           | 43:262                      | 7.350   | .007*| 0.469 (0.268–0.819)  |
|        | TC        | 230:260          | 234:262                     | 0.006   | .949| 0.992 (0.962–1.003)  |
|        | TT        | 2:260            | 0.262                       | 2.008   | .499| 0.992 (0.962–1.003)  |
| Male   | CC        | 141:131          | 129:133                     | 0.361   | .604| 1.110 (0.790–1.558)  |
|        | CT        | 18:131           | 23:133                      | 0.464   | .508| 0.796 (0.410–1.541)  |
|        | TC        | 103:131          | 114:133                     | 0.222   | .648| 0.917 (0.640–1.314)  |
| Female | CC        | 127:129          | 118:129                     | 0.170   | .721| 1.076 (0.759–1.527)  |
|        | CT        | 2:129            | 20:129                      | 13.626  | .000*| 0.100 (0.023–0.437)  |
|        | TC        | 127:129          | 120:129                     | 0.101   | .790| 1.058 (0.747–1.500)  |
|        | TT        | 2:129            | 0:129                       | 1.985   | .498| 0.985 (0.964–1.006)  |

*P < .05.
4. Discussion
In this study, the rs2301708 and rs7444370 SNPs in the KLHL3 gene were investigated as nonmodifiable risk factors for EH in a prospective case–control study in a typical Southern Han Chinese population. The rs7444370 in healthy volunteers had no obvious association with levels of blood pressure. In fact, in this study, the rs7444370 did not manifest a significant difference between the EH patients and the normotensive group; however, the relationship of SNP rs7444370 with EH showed a significant difference between the female EH patients and female subjects in the normotensive group. Our results reveal that the frequencies of the rs7444370 T allele and CT genotype in female EH patients were significantly decreased as compared to normotensive controls. However, there was no statistically significant difference in the genotype and allele frequencies of KLHL3 in male subjects in the EH and normotensive control groups. Moreover, the haplotype frequency distributions of the two SNPs of the KLHL3 gene in the EH patient group and the normotensive control group indicated that the haplotype CT of rs7444370 and rs2301708 has a protective effect in women. Our data suggest that KLHL3 rs7444370C allele, CT genotype, and the haplotype CT of rs7444370 and rs2301708 are associated with protection against EH in Chinese Han females.

KLHL3 is an important member of the BTB-containing Kelch proteins, which recruit substrates for the CRL complex. CUL3 provides the scaffold that binds to the BTB domain of KLHL3 through its N-terminus. Mutations of the KLHL3 gene have been identified to cause PHAII and is probably related to the association of mutations in WNK1 and WNK4 with PHAII. The WNK1 and WNK4 isoforms play important roles in controlling blood pressure through modulation of two homologous kinases (SPS1-related proline/alanine-rich kinase [SPAK] and oxidative stress-responsive kinase 1 [OSR1]) that phosphor- ylate and activate the Na+/Cl–cotransporter (NCC) and Na+/K+2Cl– cotransporter (NKCC) 1 and 2. KLHL3 mutations affect WNK4 ubiquitination and, thus, the WNK4 protein level through the CRL complex, which regulates blood pressure by interaction with KLHL3 and, thereby, modulates WNK ubiquitination. Moreover, mutations in WNK4 have an inhibitory effect on its interaction with KLHL3 and cause elevated blood pressure by increasing WNK4 expression. Furthermore, impaired WNK4 ubiquitination, a common pathogenic mechanism of hereditary hypertension in humans, results from mutations of WNK4 and KLHL3 in PHAII. In addition, WNK1 ubiquitination and the ensuing degradation in the disease is associated with KLHL3 mutations. It is estimated that approximately 10% of PHAII patients are affected by WNK1 or WNK4 mutations. Recently, variations in WNK1 and WNK4 have been found in EH patients, and some polymorphisms may play important roles in the increased susceptibility to EH. KLHL3 mutations regulate the expression of WNK1 and WNK4 isoforms, which regulate blood pressure. Moreover, thiazide diuretics inhibit NCC in the nephrons, where KLHL3 is expressed; thus, thiazide diuretics reverse the clinical features of hypertension, suggesting a potential link between KLHL3 and Na+/Cl–reabsorption. The physiologic adaptation of KLHL3 in low-K+–mediated induction of NCC reduces distal electronegative Na+ reabsorption, preventing further renal K+ loss but promoting increased blood pressure. Based on previous studies and this one, we inferred that KLHL3 mutations might influence vulnerability to EH. Together, the KLHL3 mutations play an important role in regulating WNK ubiquitylation and blood pressure, and the CUL3/KLHL3–WNK–SPAK/OSR1 pathway as a potential target for antihypertensive therapy.

In this study, we demonstrated KLHL3 rs7444370 maybe a potential SNP that could serve as a possible protective genetic factor against the progression of EH in Chinese Han females. However, no significant association was detected between the KLHL3 rs2301708 SNP and EH in the Chinese Han population. Our results revealed that the KLHL3 rs7444370C allele and CT genotype are associated with protection against EH in women. Furthermore, the study of haplotype frequency distributions of KLHL3 rs7444370 and rs2301708 in EH and control groups highlights that the haplotype CT has a protective effect in women.

5. Limitations
We recognize that our study has several limitations. For example, we analyzed the relationship between KLHL3 rs2301708 and rs7444370 polymorphisms with EH in the southern Chinese Han population, with a relatively small sample size. Only 522 participants, including 260 EH patients and 262 normotensive individuals, were recruited over nearly 2 years, because of the challenges in educating potential subjects about the clinical study and getting them to provide written informed consent. Another limitation was that only two SNPs within the KLHL3 gene were analyzed.

6. Future directions
Additional in-depth studies are needed to confirm the functional importance of the KLHL3 rs7444370 polymorphism in the progression of EH and to elucidate its precise role in the pathogenesis of EH. In summary, KLHL3 SNP rs7444370 could be a protective factor against the pathogenesis of females’ EH especially C allele and CT genotype. Therefore, KLHL3 is a potential regulator of blood pressure in women. Further studies are warranted to elucidate the mechanism of action of KLHL3 in regulating blood pressure or whether the gene and protein expression of KLHL3 will be altered by KLHL3 rs7444370.

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