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NR3C2 gene polymorphism is associated with risk of gestational hypertension in Han Chinese women

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Abstract

Background: The influence of genetic polymorphisms on the development of gestational hypertension (GH) is unclear. The aim of this study was to examine whether single-nucleotide polymorphisms (SNPs) of the nuclear receptor subfamily 3, group C, member 2 (*NR3C2*) genes, rs5522, rs2070951, rs5534, s2248038, and s9992256 are associated with GH in Han Chinese women.

Method: Sanger sequencing was used to analyze the genotypes of rs5522, rs2070951, rs5534, rs2248038, and rs9992256 loci of the *NR3C2* gene in 450 patients with GH and 450 healthy controls.

Results: The rs5522 dominant model (odds ratio [OR] = 1.30, 95% confidence interval [CI]: 1.13–1.47, $P < .001$) and the recessive model (OR = 1.64, 95% CI: 1.33–1.86, $P < .001$) had higher GH risk. The rs2070951 dominant model (OR = 1.18, 95% CI: 1.03–1.35, $P = .02$) had higher risk of GH, and the recessive model (OR = 1.09, 95% CI: 0.84–1.34, $P = .55$) was not significant for GH risk. The rs5534 dominant model (OR = 1.25, 95% CI: 1.09–1.43, $P = .001$) had a higher GH risk. The rs2248038 and rs9992256 sites were not significantly related to GH risk. Gene–gene interactions at the rs5522, rs2070951, and rs5534 loci affected GH risk (OR = 1.34, 95% CI: 1.12–1.64, $P < .001$).

Conclusion: The SNPs of the *NR3C2* gene rs5522, rs2070951, and rs5534 are associated with GH in Han Chinese women.

Abbreviations: BMI = body mass index, cCSC = chronic central serous chorioretinopathy, CI = confidence interval, DBP = diastolic blood pressure, GH = gestational hypertension, HR = heart rate, MDR = multifactor dimensionality reduction, MR = mineralocorticoid receptor, *NR3C2* = the nuclear receptor subfamily 3, group C, member 2, OR = odds ratio, SBP = systolic blood pressure, SNP = single-nucleotide polymorphism.

Keywords: gene–gene interactions, gestational hypertension, *NR3C2*, single-nucleotide polymorphism

1. Introduction

Gestational hypertension (GH) is a disease that is unique to pregnancy and is a major cause of maternal and perinatal death. GH can lead to the occurrence of peripheral and central vascular and cerebrovascular diseases of the mother.[1] At present, the pathogenesis of GH is still not clear, and there are no effective prevention and treatment measures in clinical practice. The main means of treatment are symptomatic treatment and termination of pregnancy. Therefore, the clinical efficacy of some patients with severe illness is poor, and the risk of death of mothers and perinatal infants is increased.[2,3]

The renin-angiotensin-aldosterone system is considered the major factor in the regulation of blood pressure, and is targeted by a variety of antihypertensive drugs.[4,5] The mineralocorticoid receptor (MR) is important for GH.[6] The MR, which is also termed the aldosterone receptor or *NR3C2*, is a member of the nuclear receptor family. It consists of 3 functional domains: the N-terminal domain, DNA domain, and C-terminal ligand domain.[7] The MR is expressed in numerous epithelial tissues, such as kidneys, salivary glands, sweat glands, and intestines, and promotes epithelial sodium reabsorption and potassium excretion to regulate electrolyte balance and blood pressure.[8,9]

The MR is encoded by the *NR3C2* gene, which is located in the q31.1 region of human chromosome 4. The gene has a length of 400 kb and includes 9 exons. The 984 amino acid protein is encoded by exons 2 to 9. *NR3C2* gene mutations are associated with the occurrence of various diseases. For example, Van et al[10] showed that the rs2070951 and rs5522 mutations in the *NR3C2* gene are significantly associated with chronic central serous chorioretinopathy (cCSC). Bogdan et al[11] found

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that mutation at the rs5522 site of the *NR3C2* gene is associated with stress sensitivity, elevated depressive symptoms, and reduced cortisol induction. Vogel et al[12] showed that the rs5534 mutation is associated with a negative memory bias in depression.

The relationship between *NR3C2* gene mutations and GH in Han Chinese women is unclear. In this study, we selected 3 single-nucleotide polymorphism (SNP) sites for the *NR3C2* gene mutations: rs5522, rs2070951, and rs5534. We also selected 2 SNP loci (rs2248038 and rs9992256) with minor allele frequency >0.05 according to the results of the 1000 genomes project of the Han Chinese population (https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/). The effects of interactions of these 5 *NR3C2* SNPs on GH in Han Chinese women were determined.

2. Materials and methods

2.1. General information

We selected 450 Han Chinese GH women hospitalized in the obstetric department of our hospital from August 2014 to October 2017 as the case group. They ranged in age from 19 to 41 years. The diagnosis of GH was based on the diagnostic criteria for hypertension in pregnancy established by the International Pregnancy Hypertension Study Society.[13] Pregnancy-induced hypertension was defined as GH (systolic blood pressure [SBP] ≥ 140 mm Hg or diastolic blood pressure [DBP] ≥ 90 mm Hg, separated by at least 6 hours and measured at least twice) after 20 weeks of pregnancy, without significant proteinuria (<0.3 g/24 h) and which returned to normal 12 weeks after delivery. Patients with essential hypertension, diabetes, and kidney disease were excluded. At the same time, 450 Chinese Han women with healthy pregnancy were recruited as the control group, aged from 19 to 42 years, excluding patients with hypertension, coronary heart disease, cerebrovascular disease, and diabetes. This study was approved by the medical ethics committee of our hospital, and all subjects signed informed consent.

2.2. Methods

Blood samples (5 mL) collected from the elbow veins of the subjects and were anticoagulated with disodium edetate. Genomic DNA was extracted using the QIA-amp DSP DNA Blood Mini Kit (Qiagen, Duesseldorf, Germany) and stored frozen at −20°C. Primer was amplified by polymerase chain reaction (PCR) according to the SNP site of the *NR3C2* gene sequence information provided in Table 1. The primers were synthesized by GENEWIZ Corp (South Plainfield, NJ). The 25 μL PCR reaction volume contained 500 ng genomic DNA template, 0.6 μL Taq DNA polymerase (5 IU/μL), 2 μL 2.5 pmol/μL dNTP, 2.5 μL buffer, 1.5 μL $MgCl_2$, and 10 pmol/μL of upstream and downstream primers. Sterile water was added to produce a final volume of 25 μL. The PCR amplification conditions were as follows: an initial denaturation at 94°C for 4 minutes, followed by 30 cycles denaturation at 94°C for 45 seconds, annealing at 60°C for 30 seconds, extension 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. After PCR, the products were purified and the genotype was analyzed by Sanger sequencing (Fig. 1).

2.3. Statistical analyses

The results for continuous variables are expressed as mean ± standard deviation. Independent sample *t* tests were used for statistical analysis between the 2 groups, and 1-way analysis of variance was used for difference analysis among the 3 groups. The Chi-squared test (χ^2) was used to compare categorical variables, which are expressed as n (%). Hardy–Weinberg equilibrium of the control group genotype was verified by the χ^2 test. The association between SNPs and disease status was determined based on the distribution of allele frequencies and genetic models (additive, dominant, and recessive models). Odds ratio (OR) and 95% confidence intervals (CIs) were used in unconditional logistic regression analysis. Age, gestational age, body mass index (BMI), SBP, DBP, heart rate (HR), 1- and 5-minute Apgar scores, and other factors were evaluated. Gene–gene interaction analyses were performed using multifactor dimensionality reduction (MDR) software (http://www.multifactordimensionalityreduction.org/). Statistical analyses were performed using SPSS 20.0 software (IBM, Chicago, IL). All tests were 2-tailed. A *P*-value < .05 indicated that the difference was statistically significant.

3. Results

3.1. Patient demographics and clinical characteristics

The study involved 450 cases of female Han Chinese patients with GH and 450 normal Han Chinese women as the control group. The demographic and clinical characteristics of the case

Table 1

SNP site information of the *NR3C2* gene.

SNP	Chromosome	MAF	Primer sequence (5′→3′)
rs5522	4:148436323	0.1505(C)	F: ACGTTGGATGCTCATGACACATGATAGGGC; R: ACGTTGGATGTTATGTCTGACTCTGGGAGC
rs2070951	4:148436862	0.2670(G)	F: TTGACCCCACCGTCTTTCCATA; R: TGTGGCTTAGCAAATGCAATTTTAGA
rs5534	4:148079934	0.2087(T)	F: TCCAGGAGTGCAGAACCTCT; R: TCCATGCACTCTCTCTCTGT
rs2248038	4:148443847	0.1505(C)	F: GCCTCACCCTCGCTTCG; R: CCGGAATCCCGGAGCTTAAC
rs9992256	4:148445141	0.2670(G)	F: AAAACTGAGCCCAAATGCACG; R: GAGGTCAGGGCCGGACTTTG

MAF = minor allele frequency, SNP = single-nucleotide polymorphism.

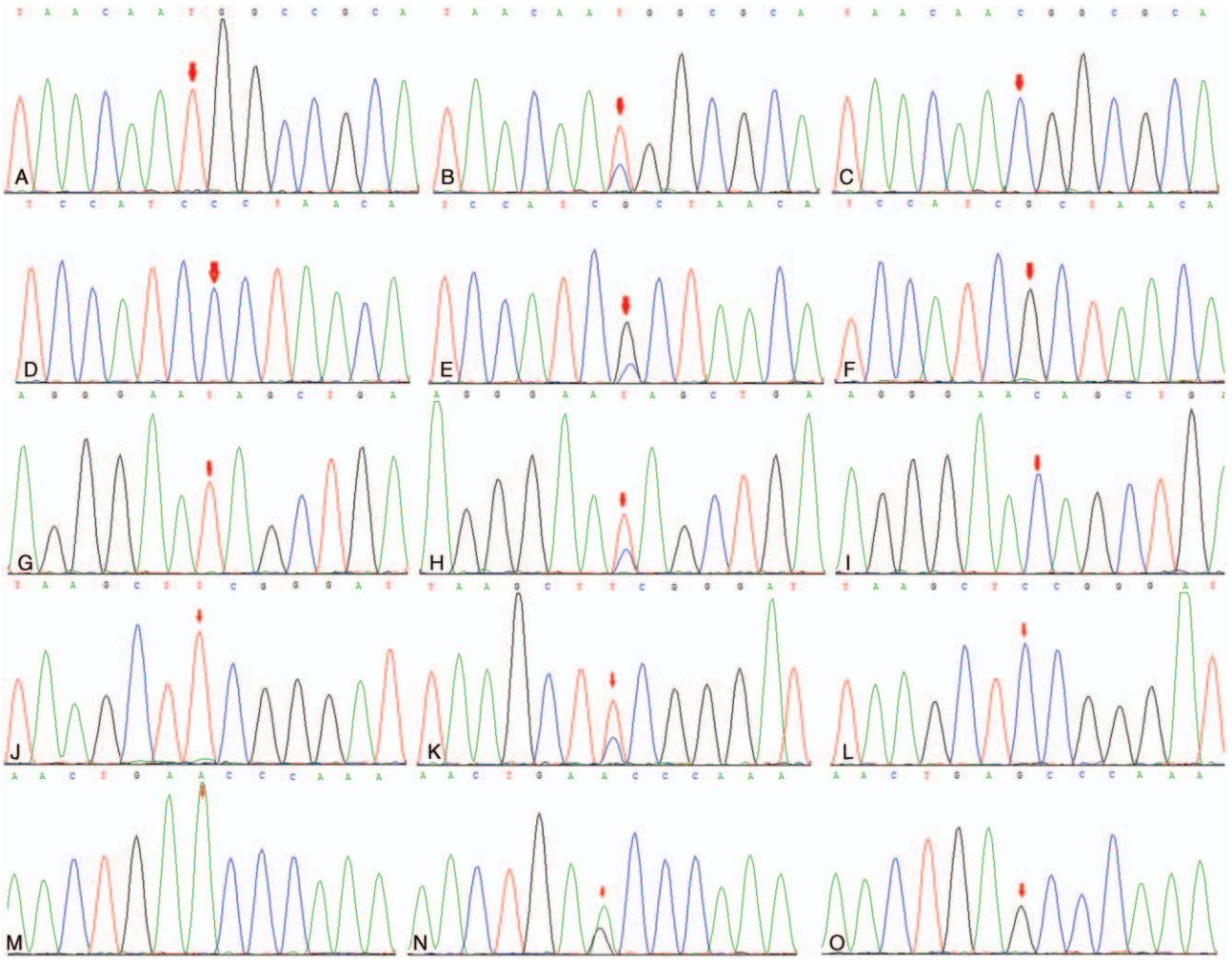

Figure 1. Sanger sequencing results. (A–C) rs5522 locus TT, TC, and CC genotypes, respectively. (D–F) rs2070951 locus CC, CG, and GG genotypes, respectively. (G–I) rs5534 locus CC, CT, and TT genotypes, respectively. (J–L) rs2248038 locus TT, TC, and CC genotypes, respectively. (M–O) rs9992256 locus AA, AG, and GG genotypes, respectively.

and control groups are shown in Table 2. During the hospitalization period, the SBP and DBP of the women in the case group were significantly higher than those of the control group. The BMI of this group of patients was also significantly higher than that of the control group ($P < .001$). The age, gestational age, neonatal weight, HR, and APGAR score (5 minutes) of the GH and control groups were not statistically different ($P > .05$).

3.2. NR3C2 gene SNPs and risk of GH

The frequencies of the genotypes of rs5522, rs2070951, rs5534, s2248038, and s9992256 SNP sites of the *NR3C2* gene displayed

Table 2

General clinical characteristics of case groups and control groups.

Characteristics	GH (n = 450)	Control (n = 450)	*P*
Age, yrs, mean ± SD	27.1 ± 5.1	26.9 ± 5.4	.57
Gestational weeks, wks, median (QL, QU)	39 (38, 40)	39 (38, 40)	.42
Neonatal weight, kg, median ± SD	3.3 ± 0.9	3.2 ± 0.8	.08
BMI, kg/m^2, mean ± SD	27.5 ± 5.3	24.2 ± 4.6	<.001
SBP, (mm Hg, mean ± SD	140.3 ± 13.8	107.4 ± 13.2	<.001
DBP, (mm Hg, mean ± SD	88.4 ± 7.1	72.7 ± 7.3	<.001
HR, (beats/min, mean ± SD	81.3 ± 9.3	82.0 ± 8.8	.25
Apgar score (1 min)	7.7 ± 1.2	8.3 ± 1.2	<.001
Apgar score (5 min)	9.0 ± 1.0	8.9 ± 1.1	.15

BMI = body mass index, DBP = diastolic blood pressure, GH = gestational hypertension, HR = heart rate, QL = lower quartile (25%), QU = upper quartile (75%), SBP = systolic blood pressure, SD = standard deviation.

Table 3

***NR3C2* gene SNP locus genotypes and allele frequencies.**

SNP	GH (n = 450)	Control (n = 450)	HWE *P*	χ^2	*P*	Adjusted OR (95% CI)	Adjusted *P*
rs5522							
TT	285 (63.3%)	337 (74.9%)	.33	23.80	<.001	1.00 (reference)	
TC	124 (27.6%)	102 (22.7%)				1.20 (1.02–1.38)	.02
CC	41 (9.1%)	11 (2.4%)				1.72 (1.39–1.96)	<.001
TC + CC	165 (36.7%)	113 (25.1%)				1.30 (1.13–1.47)	<.001
TT + TC	409 (90.9%)	439 (97.6%)				1.00 (reference)	
CC	41 (9.1%)	11 (2.4%)				1.64 (1.33–1.86)	<.001
T	694 (77.1%)	776 (86.2%)				1.00 (reference)	
C	206 (22.9%)	124 (13.8%)				1.32 (1.19–1.46)	<.001
rs2070951							
CC	224 (49.8%)	261 (58.0%)	.07	6.13	.04	1.00 (reference)	
CG	185 (41.1%)	154 (34.2%)				1.18 (1.02–1.36)	.02
GG	41 (9.1%)	35 (7.8%)				1.17 (0.90–1.45)	.26
CG + GG	226 (50.2%)	189 (42.0%)				1.18 (1.03–1.35)	.02
CC + CG	409 (90.9%)	415 (92.2%)				1.00 (reference)	
GG	41 (9.1%)	35 (7.8%)				1.09 (0.84–1.34)	.55
C	633 (70.3%)	676 (75.1%)				1.00 (reference)	
G	267 (29.7%)	224 (24.9%)				1.13 (1.01–1.24)	.03
rs5534							
CC	231 (51.3%)	281 (62.4%)	.08	12.10	.002	1.00 (reference)	
CT	175 (38.9%)	141 (31.3%)				1.23 (1.06–1.41)	.01
TT	44 (9.8%)	28 (6.2%)				1.35 (1.06–1.64)	.02
CT + TT	219 (48.7%)	169 (37.6%)				1.25 (1.09–1.43)	.001
CC + CT	406 (90.2%)	422 (93.8%)				1.00 (reference)	
TT	44 (9.8%)	28 (6.2%)				1.25 (0.99–1.49)	.07
C	637 (70.8%)	703 (78.1%)				1.00 (reference)	
T	263 (29.2%)	197 (21.9%)				1.20 (1.09–1.32)	<.001
rs2248038							
TT	331 (73.6%)	339 (75.3%)	.15	1.00	.61	1.00 (reference)	
TC	102 (22.7%)	99 (22.0%)				1.03 (0.87–1.20)	.80
CC	17 (3.8%)	12 (2.7%)				1.19 (0.79–1.56)	.43
TC + CC	119 (26.4%)	111 (24.7%)				1.05 (0.89–1.21)	.59
TT + TC	433 (96.2%)	438 (97.3%)				1.00 (reference)	
CC	17 (3.8%)	12 (2.7%)				1.18 (0.78–1.54)	.45
T	764 (84.9%)	777 (86.3%)				1.00 (reference)	
C	136 (5.1%)	123 (13.7%)				1.06 (0.92–1.20)	.42
rs9992256							
AA	242 (53.8%)	254 (56.4%)	.55	4.45	.11	1.00 (reference)	
AG	159 (35.3%)	165 (36.7%)				1.01 (0.87–1.16)	.99
GG	49 (10.9%)	31 (6.9%)				1.26 (0.99–1.51)	.05
AG + GG	208 (46.2%)	196 (43.6%)				1.06 (0.92–1.21)	.46
AA + AG	401 (89.1%)	419 (93.1%)				1.00 (reference)	
GG	49 (10.9%)	31 (6.9%)				1.25 (1.01–1.49)	.04
A	643 (71.4%)	673 (74.8%)				1.00 (reference)	
G	257 (28.6%)	227 (25.2%)				1.09 (0.98–1.20)	.12

CI = confidence interval, GH = gestational hypertension, HWE = Hardy–Weinberg equilibrium, OR = odds ratio, SNP = single-nucleotide polymorphism.

Hardy–Weinberg equilibrium ($P > .05$) (Table 3). The genotype frequencies of the rs5522 locus were significantly different between the GH group and the control group ($P < .001$). TC and CC genotype frequencies were higher in the GH group. There was significantly higher risk of GH in the dominant model (OR = 1.30, 95% CI: 1.13–1.47, $P < .001$) and in the recessive model (OR = 1.64, 95% CI: 1.33–1.86, $P < .001$). The rs5522 locus C allele was a high risk factor for GH (OR = 1.32, 95% CI: 1.19–1.46, $P < .001$) (Table 3). The frequencies of the CG and GG genotypes in rs2070951 were significantly higher in the GH group than in the control group ($P = .04$). The GH risk was significantly lower in the dominant model (OR = 1.18, 95% CI: 1.03–1.35, $P = .02$). The risk of GH was not significant in the recessive models (OR = 1.09, 95% CI: 0.84–1.34, $P = .55$), and the G allele at the rs2070951 locus was a high risk factor for GH (OR = 1.13, 95% CI: 1.01–1.24, $P = .03$) (Table 3). The frequencies of the rs5534 locus CT and TT genotype were significantly higher in the GH group ($P = .002$). The risk of GH was higher in the dominant model (OR = 1.25, 95% CI: 1.09–1.43, $P = .001$) and lower in the recessive model (OR = 1.25, 95% CI: 0.99–1.49, $P = .07$). The T allele was a high risk factor for GH (OR = 1.20, 95% CI: 1.09–1.32, $P < .001$) (Table 3). The genotype frequencies of the rs2248038 and rs9992256 locus were not significantly different between the GH group and the control group ($P = .61$ and .11, respectively). The GH risk was lower in the dominant model and the recessive model, and the 2 SNP loci were not significantly associated with GH risk (Table 3).

Table 4

***NR3C2* gene single-nucleotide polymorphism and GH risk correlation in different body mass index subjects.**

	GH (n = 450)	Control (n = 450)	OR (95% CI)	*P*
<24				
rs5522				
TT	30 (49.18%)	166 (72.49%)	1.00 (reference)	
TC/CC	31 (50.82%)	63 (27.51%)	2.16 (1.35–3.43)	.001
rs2070951				
CC	25 (40.98%)	134 (58.52%)	1.00 (reference)	
CG/GG	36 (59.02%)	95 (41.48%)	1.75 (1.08–2.86)	.02
rs5534				
CC	33 (54.10%)	146 (63.76%)	1.00 (reference)	
CT/TT	28 (45.90%)	83 (36.24%)	1.37 (0.85–2.19)	.22
rs2248038				
TT	50 (81.97%)	170 (74.24%)	1.00 (reference)	
TC/CC	11 (18.03%)	59 (25.76%)	0.69 (0.35–1.27)	.28
rs9992256				
AA	33 (54.10%)	134 (58.52%)	1.00 (reference)	
AG/GG	28 (45.90%)	95 (41.48%)	1.15 (0.71–1.85)	.64
≥24				
rs5522				
TT	255 (65.55%)	171 (77.38%)	1.00 (reference)	
TC/CC	134 (34.45%)	50 (22.62%)	1.22 (1.07–1.36)	.003
rs2070951				
CC	199 (51.16%)	127 (57.47%)	1.00 (reference)	
CG/GG	190 (48.84%)	94 (42.53%)	1.10 (0.97–1.24)	.16
rs5534				
CC	198 (50.90%)	135 (61.09%)	1.00 (reference)	
CT/TT	191 (49.10%)	86 (38.91%)	1.16 (1.02–1.31)	.02
rs2248038				
TT	281 (72.24%)	169 (76.47%)	1.00 (reference)	
TC/CC	108 (27.76%)	52 (23.53%)	1.08 (0.94–1.23)	.30
rs9992256				
AA	209 (53.73%)	120 (54.30%)	1.00 (reference)	
AG/GG	180 (46.27%)	101 (45.70%)	1.01 (0.89–1.14)	.96

CI = confidence interval, GH = gestational hypertension, OR = odds ratio.

3.3. NR3C2 gene SNP and PH risk correlation in different BMI subjects

We analyzed the correlation between *NR3C2* gene SNP and PH risk in different BMI subjects by stratified analysis. The results showed that C was carried in nonobese subjects (BMI < 24 kg/m^2) and obese subjects (BMI ≥ 24 kg/m^2). The risk of GH was significantly elevated ($P < .05$) in the gene (TC/CC) (Table 4). However, the risk of GH was significantly increased ($P < .05$) when the rs2070951 locus G allele (CG/GG) was observed in nonobese subjects (BMI < 24 kg/m^2) (Table 4). The risk of GH was significantly increased ($P < .05$) only when the rs5534 site T allele (CT/TT) was carried in obese subjects (BMI ≥ 24 kg/m^2) (Table 4). The nonobese subjects (BMI <24 kg/m^2) and obese subjects (BMI ≥ 24 kg/m^2) carry the rs2248038C allele (TC/CC) and the rs9992256 G allele (AG/GG), there was no significant change in the risk of GH ($P > .05$) (Table 4). This indicates that BMI has a significant interaction with the *NR3C2* gene rs2070951 locus and rs5534 locus.

3.4. Influence of NR3C2 gene SNP locus interaction on GH risk

The MDR method was used to analyze the interactions of the rs5522, rs2070951, rs5534, rs2248038, and rs9992256 loci of the *NR3C2* gene (Table 5, Figs. 2 and 3). The best model contained the rs5522, rs2070951, and rs5534 loci, suggesting gene–gene interactions at these loci (Table 5). Dendrogram results revealed an enhanced interaction between the rs2248038 and rs9992256 loci, and a strong interaction between the rs2070951 and rs5534 loci. The latter 2 loci displayed a strong

Table 5

Best models to analyze gene–gene interactions by multifactor dimensionality reduction.

Model	Bal. Acc. CV training	Bal. Acc. CV testing	CV consistency	*P*	OR (95% CI)
rs5522	0.6038	0.5678	9/10	.023	1.18 (1.03–1.34)
rs5522, rs5534	0.6326	0.5633	8/10	.015	1.22 (1.09–1.45)
rs5522, rs2070951, rs5534	0.6549	0.5644	10/10	<.001	1.34 (1.12–1.64)
rs5522, rs2070951, rs5534, rs9992256	0.5601	0.5322	8/10	.083	1.04 (0.98–1.32)
rs5522, rs2070951, rs5534, rs2248038, rs9992256	0.5815	0.5678	9/10	.076	1.09 (0.99–1.24)

CI = confidence interval, CV = cross-validation, OR = odds ratio.

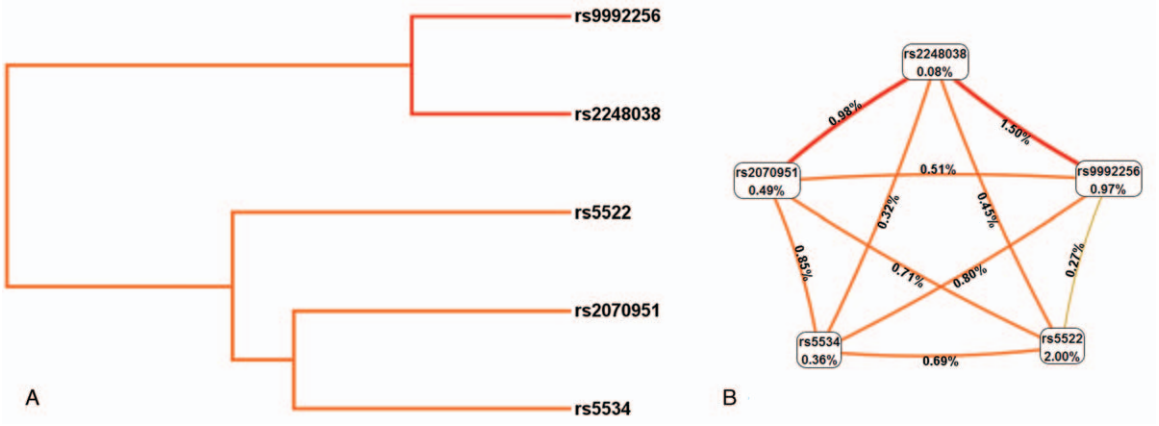

Figure 2. Hierarchical interaction graphs and interaction dendrogram. (A) In the interaction dendrogram, the red line depicts the synergy interaction and the orange line depicts weaker synergy interaction. The interaction becomes more intense moving from left to right. (B) In the hierarchical interaction graphs, entropy is indicated as a percentage at the bottom of each polymorphism, and the percentage on each line is the percentage interaction of entropy between 2 polymorphisms. The red line depicts synergy redundancy interaction and the blue line depicts redundancy interaction.

interaction with rs5522 (Fig. 2A). The Kamada–Kawaii algorithm results revealed interaction entropy of 1.50% between the rs2248038 and rs9992256 sites, 0.85% between the rs2070951 and rs5534 sites, 0.71% between the rs2070951 and rs5522 sites, and 0.69% between the rs5534 and rs5522 sites (Fig. 2B). The MDR method was also used to analyze the effect of the *NR3C2* gene SNP locus gene–gene interaction on GH risk. The best models for predicting GH risk were rs5522, rs2070951, and rs5534 (OR = 1.34, 95% CI = 1.12–1.64, $P < .001$) (Fig. 3).

3.5. Correlation between NR3C2 gene SNPs and blood pressure

The SBP and DBP were significantly higher in the GH group than in the control group (both $P < .001$; Fig. 4A and C). Analysis of the association between *NR3C2* SNPs and SBP and DBP revealed that the SBP and DBP of the rs5522, rs2070951, and rs5534 SNPs were significantly higher than wild-type (all $P < .05$; Fig. 4B and D). There were no significant differences between the SBP and DBP of the rs2248038 and rs9992256 genotypes (all $P > .05$; Fig. 4B and D).

4. Discussion

We found that the *NR552C2* gene rs5522, rs2070951, and rs5534 SNPs were associated with increased risk of GH in Chinese Han women by case–control study. In addition, the results of this study also found that gene–gene interactions in rs5522, rs2070951, and rs5534 sites have an impact on GH risk.

The RAAS gene is a well-recognized candidate for hypertension, and 5 key genes are involved in its cascade reaction, namely renin gene, angiotensinogen gene, angiotensin converting enzyme gene, angiotensin II type 1 receptor gene, and CYP11B2 gene.[14–16] Most of the previous studies have focused on the 1st half of RAAS. With the deepening of research, it has now been recognized that aldosterone in the latter part of the system plays an important role in the development of hypertension. Aldosterone affects blood pressure levels by acting on endothelial cells to regulate water and electrolyte metabolism in the body.[17]

The MR is the main receptor of aldosterone downstream in the renin-angiotensin-aldosterone system. Generally, MR activation by aldosterone promotes reabsorption of sodium in the epithelium and excretion of potassium, thereby regulating electrolyte balance.[18] Structurally, MR protein resembles a typical nuclear steroid hormone receptor, with an N-terminal domain that activates gene transcription, an intermediate domain that binds to DNA sites, and a C-terminal domain that binds to a ligand.[19] As a ligand-activated transcription factor, MR induces or inhibits specific target genes by binding to various coactivators and coreceptor-regulated hormone-responsive DNA elements.[20]

There are multiple polymorphic sites associated with disease progression. Results of karyotyping and fluorescent in situ hybridization analyses of type 1 pseudohyperaldosteronism patients may be related to the disruption of the chromosome structure of *NR3C2*.[21] This study found that SNPs at rs5522, rs2070951, and rs5534 were associated with GH risk. A case–control study found that the frequencies of these mutant alleles were significantly higher in the GH group than of the control group. We further analyzed the positions of these SNPs on the *NR3C2* gene and found that the rs5522 locus is located on exon 2 of the *NR3C2* gene and the rs2070951 (MR c.2C>G) polymorphism is located 2 nucleotides upstream of the 1st translation start site.[22] The exact effect of this polymorphism on MR remains unclear.

There is currently no consensus on the effect of this polymorphism on hypertension and the cardiovascular system. The results of this study show that the site polymorphism is associated with the risk of GH. Ritter et al[23] demonstrated the association of the rs5522 SNP with the resistant hypertension, while the rs2070951 locus c.-2C>G mutation was not associated. A study of serum aldosterone levels found a statistically significant difference in the levels between different genotypes of the rs5522 locus, but not between different genotypes of the rs2070951 locus. Van et al[10] reported the low expression of MR in the rs2070951 locus G allele, with higher SBP in male carriers

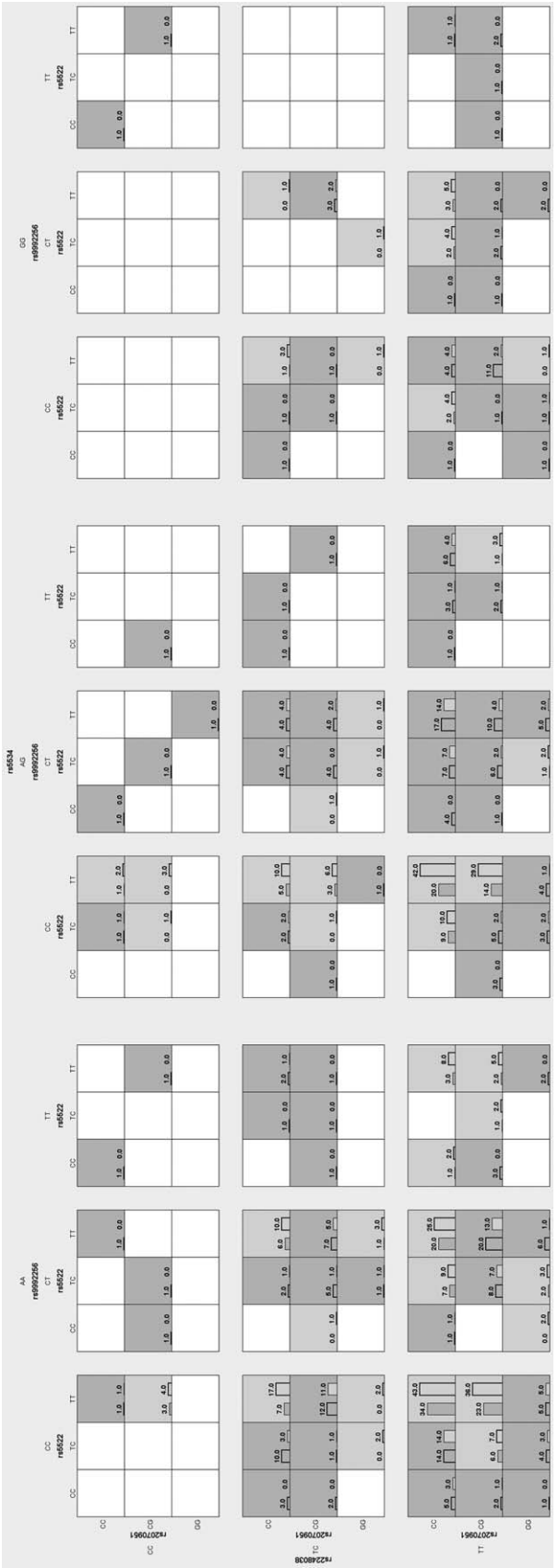

Figure 3. Distribution of high risk and low risk of gestational hypertension in the best model. Dark gray and light gray boxes depict the high- and low-risk factor combinations, respectively. Left and right bars within each box represent case and control, respectively. The heights of the bars are proportional to the sum of the samples in each group. The patterns of high- and low-risk cells differ across each of the different multilocus dimensions, which is evidence of gene–gene interaction.

of the GG genotype, consistent with the results of this study. The results substantiate the influence of the rs5522 locus polymorphism on MR. The impact of the rs2070951 locus on MR remains contentious. We believe that the reason may be related to ethnic differences. The rs5534 locus is located on exon 9 of the *NR3C2* gene. Vogel et al[12] reported that rs5534 locus was associated with negative memory bias and life stress. In addition, this encoded SNP in exon 9 of the *NR3C2* gene was associated with decreased efficiency of the upstream binding site of the hsa-miR-383 microRNA in 2 human cell lines.[24] In vitro, the A allele at the rs5534 locus increased microRNA-induced inhibition of MR expression.[24] Therefore, this SNP may be a sign of impaired expression of MR, which may lead to dysfunction of cortisol regulation after stress.

The results of this study showed that the C>T mutation at the rs5534 locus resulted in an increased risk of GH. The reason for this may be that the C>T mutation at the rs5534 site was located in the 3′ untranslated region, this site mutation may affect the regulation of *NR3C2* expression by miRNAs.[24] In addition, this study also included the rs2248038 and rs9992256 SNPs sites located in the intron, in the noncoding region of the *NR3C2* gene. Whether they are involved in regulation of transcription and translation is still unclear. There are few studies on these 2 SNP loci, but the available data indicate that the mutation rate is higher in the Han Chinese population (Table 1). Therefore, it is also necessary to study whether these 2 SNP loci are related to the risk of GH. From the results of this study, it can be seen that the rs2248038 and rs9992256 SNPs are not high-order factors for GH occurrence. Although there is a high mutation rate in the Han Chinese population, this polymorphism does not affect the structure and function of MR, and does not significantly affect SBP and DBP.

We also analyzed the effect of the *NR3C2* gene SNP locus gene–gene interaction on GH risk. Gene–gene interactions at the rs5522, rs2070951, and rs5534 loci affected GH risk. The gene interactions involving the 3 SNP loci increased the risk of GH by 1.34 times, which was significantly higher than the impact of each SNP locus on GH risk, indicating that the effect of these 3 SNP loci on the GH risk has a significant additive effect.

There are some shortcomings in this study. This study failed to analyze the effects of these SNPs and MR structure and function at the molecular level. The lack of a suitable model is the main reason. In addition, the study is also a pilot study. There is not much research about genetic variation and GH in Han Chinese women due to the small numbers of women who have been studied. The number of individual SNP loci is also small, which could affect the results. The accuracy of the data could benefit from analysis of more women.

In summary, this is the first report that *NR3C2* gene rs5522, rs2070951, rs5534, s2248038, and s9992256 SNPs are associated with GH in Han Chinese women. SNPs at rs5522, rs2070951, and rs5534 in the *NR3C2* gene are associated with risk of GH in Han Chinese women. The intrinsic mechanism may be related to changes in the structure and function of MR. Further research at the molecular level is necessary to confirm our findings.

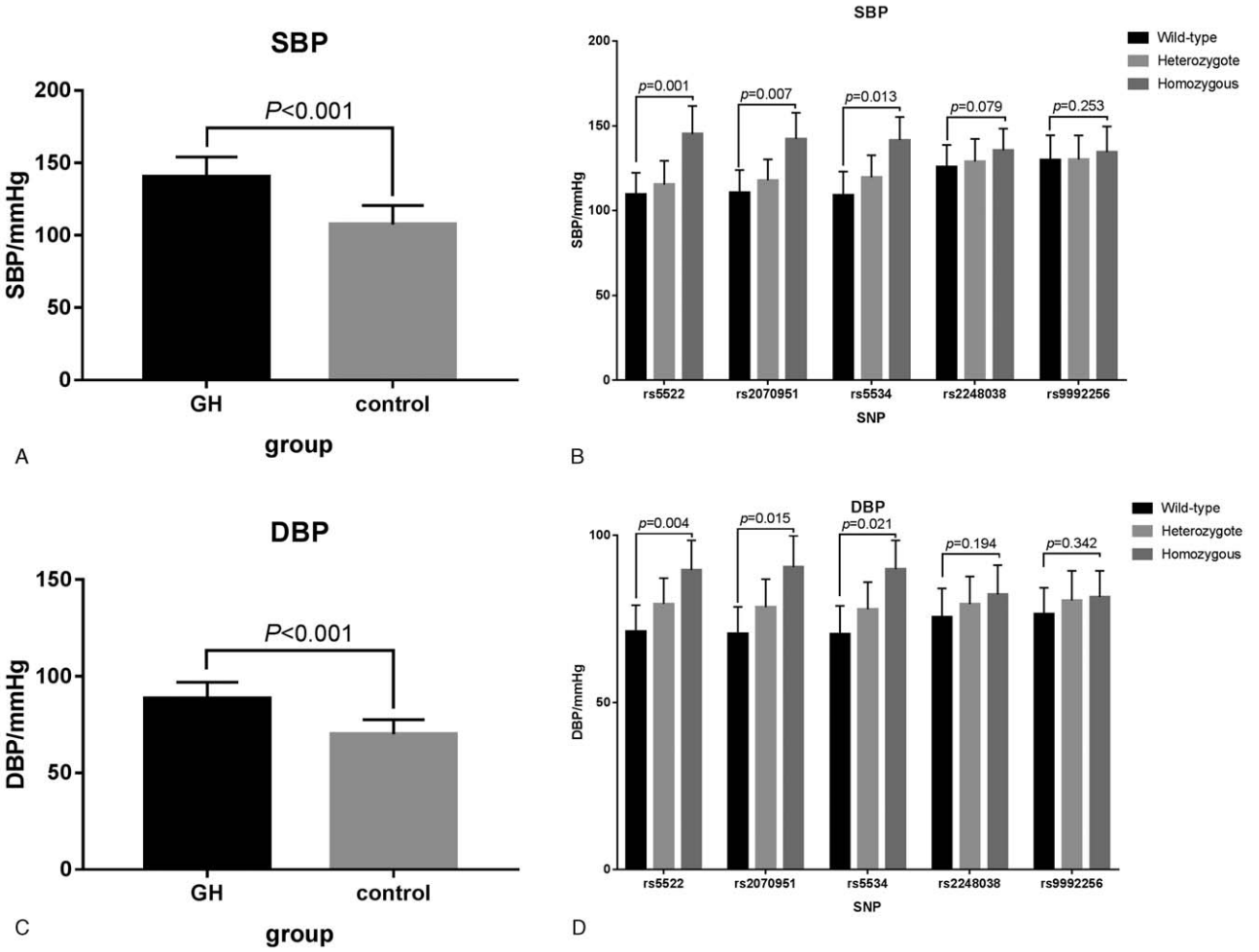

Figure 4. Comparisons of systolic blood pressure (SBP) and diastolic blood pressure (DBP) levels between different groups. (A) SBP levels of the gestational hypertension group and the control group. (B) SBP levels among subjects with the single-nucleotide polymorphism (SNP) locus of the *NR3C2* gene. (C) DBP levels of the GH group and the control group. (D) DBP levels among subjects with the SNP locus of the *NR3C2* gene.

Author contributions

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Methodology: Jianyun Xu.
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Writing – review & editing: Wenying Jiang.

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