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Associations of genetic variations in the M3 receptor with salt sensitivity, longitudinal changes in blood pressure and the incidence of hypertension in Chinese adults

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Abstract

Recent studies have reported the role of the M3 muscarinic acetylcholine receptor (M3R), a member of the G-protein coupled receptor superfamily, encoded by the *CHRM3* gene, in cardiac function and the regulation of blood pressure (BP). The aim of this study was to investigate the associations of *CHRM3* genetic variants with salt sensitivity, longitudinal BP changes, and the development of hypertension in a Chinese population. We conducted a chronic dietary salt intervention experiment in a previously established Chinese cohort to analyze salt sensitivity of BP. Additionally, a 14-year follow-up was conducted on all participants in the cohort to evaluate the associations of *CHRM3* polymorphisms with longitudinal BP changes, as well as the incidence of hypertension. The single nucleotide polymorphism (SNP) rs10802811 within the *CHRM3* gene displayed significant associations with low salt-induced

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changes in systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP), while rs373288072, rs114677844, and rs663148 exhibited significant associations with SBP and MAP responses to a high-salt diet. Furthermore, the SNP rs58359377 was associated with changes in SBP and pulse pressure (PP) over the course of 14 years. Additionally, the 14-year follow-up revealed a significant association between the rs619288 polymorphism and an increased risk of hypertension (OR = 1.74, 95% CI: 1.06-2.87, $p = .029$). This study provides evidence that *CHRM3* may have a role in salt sensitivity, BP progression, and the development of hypertension.

KEYWORDS
gene polymorphism, hypertension, M3 muscarinic acetylcholine receptor, salt, salt sensitivity

1 | INTRODUCTION

Hypertension, a prevalent chronic disease, is a significant risk factor for cardiac-cerebrovascular and chronic kidney disease.[1] In addition to intricate physiological systems, blood pressure (BP) is influenced by an interplay of genetic and environmental factors.[2] Of all environmental factors, high dietary salt intake has been identified as the most influential determinant of hypertension.[3,4] Moreover, individuals exhibit varying BP responses that correspond to their salt intake, leading to the concept of salt sensitivity.[5,6] Salt sensitivity is characterized by elevated BP with high salt intake and lowered BP with low salt intake, and it has been associated with an increased risk of cardiovascular events as well as being an independent risk factor for renal diseases.[7–9] Although population studies have suggested that genetic variations within individuals may contribute to the variability in BP response to salt intake,[10–12] the precise genetic mechanisms underlying salt sensitivity of BP remain incompletely understood.

The M3 muscarinic acetylcholine receptor (M3R) is one of the five muscarinic receptor subtypes (M1-5) in the G-protein-coupled receptor family, which utilize second messengers to facilitate signal transductions.[13,14] M3R is widely distributed in the human heart, has an inotropic effect, and plays vital roles in various cardiac functions, including the regulation of heart rate, mitigation of myocardial ischemic injury, facilitation of cell-to-cell communication, and prevention of atrial fibrillation.[15,16] In addition, M3R is expressed in the endothelial cell layer and smooth muscle cells of the vascular system, where it mediates vasodilation and vasoconstriction.[17,18] Moreover, M3R is involved in the regulation of BP. Previous study has shown that acetylcholine acts on the gastrointestinal M3R to increase afferent vagal activity, which decreases sympathetic nervous activity by autonomic reflex, suppressing noradrenaline release and lowering BP.[19]

CHRM3, the gene encoding M3R, is located on chromosome 1q43. It consists of a single exon and contains over 1100 single nucleotide polymorphisms (SNPs), making it the most polymorphic gene among the muscarinic receptors.[20,21] A growing body of evidence suggests that missense mutations in the *CHRM3* gene are functionally associated with increased BP due to enhanced signaling and resensitization of M3R. These mutations also promote the production of epinephrine.[22–24] Additionally, animal studies have established a connection between the *CHRM3* gene and salt-sensitive hypertension. In the Dahl salt-sensitive (DSS) rat model, elevated M3R activity was linked to high BP, while deletion of *CHRM3* gene reduced salt-induced hypertension in both male and female rats.[25] Despite available data, the impact of M3R and the *CHRM3* gene on BP salt sensitivity in humans remains undetermined.

Therefore, in this study, we conducted a chronic salt intervention experiment in a previously established Chinese cohort to investigate the relationship between *CHRM3* genetic variations and salt-induced changes in BP. Furthermore, a 14-year follow-up was performed to examine the relationship between the *CHRM3* gene, long-term BP progression, and the development of hypertension.

2 | METHODS

2.1 | Study cohort

We utilized data from the Baoji Salt-Sensitive Study cohort established in 2004, which was a family-based longitudinal study involving 514 adults from 124 families residing in seven villages located in Baoji City, Shaanxi Province, China. We included participants of Han ethnicity aged 18–60 years with a systolic blood pressure (SBP) range of 130–160 mmHg and diastolic blood pressure (DBP) range of 85–100 mmHg, who were not under antihypertensive treatment, as probands for this study. Additionally, we recruited their parents, siblings from two-generation families, spouses, and offspring from three-generation families. Individuals with secondary hypertension, severe cardiovascular disease or diabetes mellitus, liver or renal dysfunction, alcohol abuse, or pregnancy were excluded from the study. Data including social demographic survey (age, sex, education, and occupation), levels of physical activity (almost no, light, moderate, and heavy), and physical examination with anthropometric measurements were collected by trained physicians. A total of 333 non-parental participants were enrolled in a highly controlled dietary salt intervention trial to investigate the relationship between variations in the *CHRM3* gene and salt sensitivity of BP. The detailed methodology has been

outlined in previous studies.[26–28] Briefly, the salt intake intervention consisted of three phases. The initial phase involved a 3-day baseline observation period during which participants completed questionnaires and underwent physical examinations. The second phase lasted for 7 days and implemented a low-salt diet, with participants instructed to consume 3 g of sodium chloride or 51.3 mmol of sodium per day. Subsequently, the final phase also spanned 7 days and introduced a high-salt diet, with participants instructed to consume 18 g of sodium chloride or 307.8 mmol of sodium per day. All meals were provided at a designated restaurant, ensuring strict control over the salt content. Additionally, participants were strictly prohibited from consuming any additional salt during the entirety of the dietary intervention period.

To investigate the potential association between genetic variations and the long-term BP progression and the development of hypertension, all participants were followed up in 2009, 2012, and 2018. During each follow-up, BP measurements were taken three times a day, population characteristics were examined, and blood samples were collected for biochemical analysis and genotyping. The methodology for these procedures has been previously described in detail.[26–28]

2.2 | Measurement of BP and definitions

Participants were instructed to abstain from smoking, consuming alcohol, or engaging in strenuous exercise prior to BP measurements. They were asked to sit and rest for 5 min before the measurements were taken. The trained staff used a random-zero sphygmomanometer (Hawksley & Sons Ltd, Lancing, UK) to measure BP three times a day on the same arm, with intervals of 1–2 min between each measurement. BP measurements were conducted daily throughout the 3-day baseline period, the last three days of the 7-day salt intake trial, and during the follow-up periods.

Hypertension was defined as SBP ≥ 140 mmHg, and/or DBP ≥ 90 mmHg, and/or current use of antihypertensive medication. Pulse pressure (PP) was defined as SBP minus DBP. Mean arterial pressure (MAP) was defined as DBP plus 1/3PP. To intuitively evaluate the effect of salt intake on BP, the responses were calculated as follows: BP response to low-salt intake was calculated as BP on low-salt intake minus BP at baseline, and the response to high-salt intake was calculated as BP on high-salt intake minus BP on low-salt intake.[26–29] To determine salt sensitivity (SS), the average MAP of the low-salt period was subtracted from the average MAP of the high-salt period. SS was defined as an salt-induced increase in MAP of ≥5 mmHg and salt resistance (SR) as an increase of < 5 mmHg.[30,31]

2.3 | Blood biochemistry

At the end of each intervention phase, professionals collected peripheral blood samples from participants who had undergone overnight fasting. The blood samples were promptly centrifuged and stored at −80°C for future analysis. Fasting blood glucose, total cholesterol, triglyceride, and high-density lipoprotein levels were automatically measured using an automatic biochemical analyzer (Hitachi, Tokyo, Japan).

2.4 | SNP selection and genotyping

The Genome Variation and National Center for Biotechnology Information (NCBI) server were utilized to screen the SNPs of the *CHRM3* gene. The screening criteria involved the following parameters: 1) adherence to the Hardy-Weinberg equilibrium (HWE) with a *p*-value of at least .05 for the target SNP frequency distribution; 2) a minor allele frequency (MAF) of no less than 0.05; and 3) a minimum linkage disequilibrium coefficient R^2 of 0.8. Thirteen *CHRM3* SNPs (rs10802764, rs997148, rs12034970, rs6429147, rs6692904, rs75804766, rs373288072, rs619288, rs58359377, rs114677844, rs663148, rs10802811, and rs4072234) were selected and included in subsequent analyses based on these criteria. Genomic DNA was extracted from whole blood samples using the GOLDMAG Whole Blood Genomic DNA Purification Kit (Golden Magnetic Nano-Biotechnology Co. Ltd. Xi'an, China). All genotyping tests were conducted at CapitalBio (CapitalBio Corp, Beijing, China).

2.5 | Statistical analyses

Continuous variables were reported as mean ± standard deviation (SD), while categorical variables were presented as frequency and percentage. Repeated measures one-way analysis of variance (ANOVA) was employed to compare the indicators across each phase of the intervention. Plink software (http://zzz.BWh.Harvard.Edu/Plink/) was used to assess Mendelian consistency, Hardy-Weinberg equilibrium (HWE), and minor allele frequency (MAF) of each parental SNP. Mixed linear regression models, constructed using Plink software, were utilized to analyze the associations between SNPs and BP responses to salt intake, as well as long-term BP changes, under different genetic models (additive, dominant, and recessive). Additionally, the lme4 R package was used to build a generalized linear mixed model for examining the relationship between the SNPs and the development of hypertension. To ensure accurate assessment of hypertension incidence during follow-up, 51 participants with hypertension at baseline were excluded from the study. Statistical significance was determined at a threshold of $p < .05$. Age, sex, body mass index, baseline BP, baseline urinary sodium and potassium, level of physical activity, and family correlation were adjusted as random effects in each model and multiple comparisons were further conducted.

3 | RESULTS

3.1 | Characteristics of the study participants during the dietary salt intervention

Baseline characteristics of the study participants (N = 514) and BP responses to low- and high-salt diets in 333 non-parental participants are shown in Table 1. Initially, the SBP and DBP of the probands were higher in comparison to their siblings, spouses, and offspring,

TABLE 1 Characteristics of study participants during dietary salt intervention trial.

	Probands	Siblings	Spouses	Offspring	Parents
Participants (no.)	99	167	18	49	181
Male (%)	69.7	49.1	26.3	49.0	48.4
Age, years	41.8 ± 8.4	39.8 ± 7.4	47.4 ± 6.1	23.3 ± 6.9	66.1 ± 8.3
BMI, kg/m^2	23.0 ± 2.8	22.2 ± 2.9	23.1 ± 4.7	20.1 ± 2.7	20.4 ± 2.6
Level of physical activity (no., %)					
Almost no	11 (11.1)	28 (16.8)	9 (50.0)	3 (6.1)	28 (15.5)
Light	27 (27.2)	39 (23.4)	2 (11.1)	17 (34.7)	51 (28.2)
Moderate	25 (25.3)	51 (30.5)	3 (16.7)	10 (20.4)	48 (26.5)
Heavy	36 (36.4)	49 (29.3)	4 (22.2)	19 (38.8)	54 (29.8)
BP at baseline, mmHg					
SBP	120.9 ± 12.5*	107.6 ± 11.1	108.6 ± 12.2	102.7 ± 10.7	123.2 ± 21.3
DBP	79.0 ± 8.3*	70.1 ± 8.1	70.6 ± 6.9	63.4 ± 8.9	70.5 ± 10.5
MAP	93.0 ± 9.0*	82.6 ± 8.7	83.3 ± 7.9	76.5 ± 9.2	88.0 ± 13.1
BP response to low-salt diet, mmHg					
SBP	111.7 ± 10.0*†	103.4 ± 9.1†	102.5 ± 7.7†	100.3 ± 9.4†	–
DBP	72.8 ± 9.3*†	66.4 ± 7.7†	67.1 ± 5.8†	60.7 ± 8.3†	–
MAP	85.7 ± 9.0*†	78.7 ± 7.6†	78.9 ± 5.4†	73.9 ± 8.3†	–
SBP change	−8.65 ± 9.52*	−3.90 ± 5.41	−6.15 ± 7.88	−2.38 ± 4.79	–
DBP change	−6.00 ± 6.71*	−3.64 ± 4.83	−3.48 ± 6.36	−2.70 ± 5.21	–
MAP change	−6.88 ± 7.07*	−3.73 ± 4.55	−4.37 ± 6.52	−2.59 ± 4.56	–
BP response to high-salt diet, mmHg					
SBP	118.9 ± 11.2*‡	108.5 ± 11.1‡	108.4 ± 10.9‡	102.0 ± 10.0‡	–
DBP	76.2 ± 8.1*‡	68.7 ± 9.3‡	68.6 ± 7.5	60.9 ± 8.3	–
MAP	90.4 ± 8.5*‡	82.0 ± 9.5‡	81.9 ± 8.0‡	74.6 ± 8.4	–
SBP change	7.16 ± 7.40*	5.09 ± 6.50	5.93 ± 7.90	1.72 ± 4.07	–
DBP change	3.49 ± 7.33*	2.29 ± 5.73	1.51 ± 4.69	0.22 ± 4.52	–
MAP change	4.71 ± 6.86*	3.22 ± 5.60	2.98 ± 5.61	0.72 ± 3.79	–

Data are mean ± SD or percentage.
Abbreviations: BMI, body mass index; BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure.
*$p < .05$ versus the siblings, spouses or offspring.
†$p < .05$ versus the baseline period.
‡$p < .05$ versus the low-salt period.

but lower than that of their parents. During the salt intervention experiment, significant changes in BP were observed at each stage. Notably, BP displayed a downward trend during the low-salt period, and conversely, an upward trend during the high-salt period ($p < .05$). In addition, after low-salt intervention, the changes in SBP, DBP, and MAP in hypertensive persons were greater than those in persons with normal BP (all $p < .05$, Table S1). However, no significant differences were observed in BP changes between hypertensive persons and normotensive individuals after the high-salt intervention.

Urinary sodium and potassium excretion at each stage are shown in Table 2. A significant reduction in urinary sodium excretion was observed during the low-salt phase in comparison to the baseline phase. Conversely, a significant elevation in urinary sodium excretion was observed following the high-salt diet ($p < .05$). These findings suggest that the participants exhibited excellent compliance with the prescribed salt intake levels.

3.2 | Association analyses for CHRM3 SNPs and BP responses to salt intervention

As shown in Table 3, the genotype distribution frequencies of each SNP were consistent with Hardy-Weinberg equilibrium (HWE-$p > .05$), indicating genetic equilibrium within the tested population.

The associations of individual SNPs in the *CHRM3* gene with salt-induced BP changes are shown in Table 4, with results corrected for multiple adjustments. During the low-salt intake period, SNP rs10802811 demonstrated significant associations with SBP, DBP, and

TABLE 2 Effects of dietary intervention on urinary sodium and potassium excretions.

	Probands	Siblings	Spouses	Offspring
Baseline				
24 h Urinary sodium, mmol	225.1 ± 11.6	213.8 ± 16.4	218.3 ± 20.8	205.4 ± 23.4
24 h Urinary potassium, mmol	36.8 ± 10.8	38.1 ± 9.13	39.4 ± 12.3	35.8 ± 15.7
Low-salt intervention				
24 h Urinary sodium, mmol	54.9 ± 11.3*	53.6 ± 9.8*	52.8 ± 13.5*	58.2 ± 15.1*
24 h Urinary potassium, mmol	35.4 ± 8.9	39.8 ± 7.6	34.2 ± 6.7	40.5 ± 9.8
High-salt intervention				
24 h Urinary sodium, mmol	317.0 ± 21.8#	304.4 ± 28.6#	318.6 ± 20.5#	298.4 ± 25.1#
24 h Urinary potassium, mmol	43.4 ± 13.9	39.6 ± 9.1	40.2 ± 8.3	41.1 ± 14.5

Continuous variables are expressed as mean ± SD.
*$p < .05$ versus the baseline levels.
#$p < .05$ versus the low-salt intervention.

TABLE 3 Information of CHRM3 SNPs included in the study.

SNP	Position	Region	Alleles (Major/Minor)	MAF	HWE-P[a]
rs10802764	239581417	intergenic	C/T	0.408	.644
rs997148	239757346	intergenic	A/G	0.130	.259
rs12034970	239793865	intronic	A/G	0.082	1
rs6429147	239794794	intronic	C/G	0.165	.781
rs6692904	239816532	intronic	A/C	0.190	.379
rs75804766	239870889	ncRNA_exonic	A/G	0.081	.354
rs373288072	239931799	intronic	C/T	0.374	.437
rs619288	239958668	intronic	T/C	0.207	.533
rs58359377	239979730	intronic	A/G	0.385	.271
rs114677844	240018535	intronic	G/A	0.057	1
rs663148	240042944	intronic	T/C	0.058	1
rs10802811	240048652	intronic	A/G	0.243	.244
rs4072234	240073226	UTR3	T/C	0.497	1

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency; SNP, single nucleotide polymorphism.
[a]Parental generation.

MAP responses. The polymorphism rs619288 exhibited a significant correlation with DBP response, while rs58359377 showed a significant association with PP response. In the high-salt intake period, SNP rs373288072 was significantly associated with SBP, DBP, and MAP responses, whereas rs10802811 demonstrated associations with DBP, MAP, and PP responses. Additionally, rs114677844 and rs663148 were found to be significantly associated with SBP and MAP responses.

3.3 | Characteristics of the study participants during longitudinal follow-up

The demographic and clinical characteristics of participants at baseline (2004) and three follow-up periods (2009, 2012, and 2018) are shown in Table 5. Among the 514 eligible participants, 102 (19.8%) were lost to follow-up in 2009, 56 (13.6%) were lost in 2012 and 59 (16.6%) were lost in 2018. After a 14-year follow-up, steady increases were observed in SBP by 21.2 mmHg, DBP by 7.9 mmHg, and MAP by 12.3 mmHg. Out of the total participants, 160 individuals (53.9%) who were initially non-hypertensive at baseline developed hypertension over the 14-year period. In addition, compared to salt resistant individuals, salt sensitive participants were more likely to have high BP and develop hypertension (all $p < .05$, Table S2).

3.4 | Association analyses for CHRM3 SNPs with long-term changes in BP and the incidence of hypertension

Table 6 presents the relationship between the 13 selected SNPs in the *CHRM3* gene and the long-term BP changes over different study periods: 5 years (2004-2009), 8 years (2004-2012), and 14

TABLE 4 The association between CHRM3 SNPs and BP responses to dietary salt intervention.

		SBP response		DBP response		MAP response		PP response	
SNP	Allele	β	p	β	p	β	p	β	p
Low-salt intervention									
rs10802764	C	−0.054	.474	−0.050	.513	−0.056	.461	−0.021	.781
rs997148	A	0.101	.424	0.121	.330	0.123	.328	0.009	.943
rs12034970	A	−0.104	.461	0.024	.865	−0.028	.841	−0.164	.241
rs6429147	C	0.076	.470	0.024	.823	0.048	.649	0.077	.461
rs6692904	A	−0.092	.363	−0.102	.312	−0.107	.291	−0.017	.868
rs75804766	A	−0.079	.580	0.004	.976	−0.031	.830	−0.110	.436
rs373288072	C	0.049	.546	−0.076	.346	−0.030	.712	0.145	.071
rs619288	T	0.014	.889	0.759	**.018**[b]	−0.004	.972	0.034	.735
rs58359377	A	0.128	.109	0.008	.925	0.060	.459	0.164	**.039**
rs114677844	G	−0.096	.546	0.027	.864	−0.023	.886	−0.158	.320
rs663148	T	−0.120	.446	0.007	.965	−0.047	.768	−0.169	.283
rs10802811	A	−0.194	**.032**	−0.292	**.001**	−0.276	**.002**	0.044	.628
rs4072234	T	0.004	.957	0.087	.273	0.060	.454	−0.085	.281
High-salt intervention									
rs10802764	C	−0.004	.959	0.002	.983	−0.001	.996	−0.007	.923
rs997148	A	−0.156	.211	−0.191	.130	−0.192	.127	0.024	.847
rs12034970	A	0.065	.644	−0.128	.365	−0.064	.652	0.247	.080
rs6429147	C	0.640	**.048**[b]	0.019	.855	0.035	.742	0.053	.618
rs6692904	A	0.092	.364	0.173	.086	0.155	.125	−0.089	.377
rs75804766	A	0.007	.959	−0.148	.299	−0.100	.484	0.194	.175
rs373288072	C	−0.333	**.036**[b]	−0.318	**.046**[b]	−0.347	**.029**[b]	−0.088	.279
rs619288	T	−0.033	.742	0.055	.584	0.026	.800	−0.113	.261
rs58359377	A	0.006	.938	−0.026	.745	−0.016	.845	0.041	.612
rs114677844	G	−1.493	**.011**[b]	−0.206	.197	−1.277	**.029**[b]	0.048	.767
rs663148	T	−1.493	**.011**[b]	−0.212	.179	−1.277	**.029**[b]	0.079	.617
rs10802811	A	0.071	.436	0.332	**.003**[a]	0.200	**.027**	−0.212	**.020**
rs4072234	T	−0.042	.594	−0.030	.711	−0.037	.645	−0.021	.790

Associations that were not significant under any model (additive, dominant or recessive) are presented as *p* values under additive model.

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.

Bold values emphasize that the *P*-values are less than 0.05.

[a]dominant model.

[b]recessive model.

years (2004-2018). During the 5-year period, SNP rs6429147 displayed significant associations with changes in SBP, MAP and PP, while rs12034970 showed an association with change in DBP. Over the 8-year period, SNP rs10802764 exhibited significant associations with changes in SBP and PP, whereas rs58359377 significantly correlated with change in DBP. Throughout the 14-year period, SNP rs58359377 demonstrated associations with changes in SBP, MAP and PP, whereas rs10802811 significantly correlated with change in PP. In addition, the associations of *CHRM3* SNPs with the incidence of hypertension during the follow-up periods are presented in Table 7. SNP rs6429147 was significantly associated with the incidence of hypertension at both the 5-year and 8-year follow-ups. After 14 years of follow-up, SNP rs10802764 was associated with a decreased risk of hypertension (OR = 0.63, 95% CI: 0.43-0.92, $p = .017$) after adjusting for multiple clinical factors, while rs619288 was significantly associated with an increased risk of hypertension (OR = 1.74, 95% CI: 1.06-2.87, $p = .029$), suggesting that the *CHRM3* gene may be involved in the development of hypertension.

4 | DISCUSSION

Based on our dietary intervention trial, we discovered several novel SNPs within the *CHRM3* gene that demonstrate significant

TABLE 5 Participant characteristics during follow-up of the longitudinal study.

Characteristics	Baseline in 2004	Follow-up in 2009	Follow-up in 2012	Follow-up in 2018
Participant (Male, no.)	514 (267)	412 (208)	356 (185)	297 (155)
Age, years	48.6 ± 19.8	53.3 ± 14.2	56.6 ± 19.0	62.3 ± 12.1
BMI, kg/m^2	22.2 ± 3.1	22.4 ± 3.3	23.6 ± 3.5	24.6 ± 3.7
SBP, mmHg	115.2 ± 17.6	120.0 ± 17.3	129.6 ± 18.7	136.4 ± 17.4
DBP, mmHg	71.3 ± 10.0	75.8 ± 10.4	77.9 ± 10.9	79.2 ± 11.2
MAP, mmHg	86.0 ± 11.5	90.5 ± 11.7	95.1 ± 11.9	98.3 ± 12.0
Fasting glucose, mg/dL	86.9 (80.9-94.4)	91.5 (86.0-99.1)	92.6 (86.7-100.8)	90.8 (84.8-97.5)
Total cholesterol, mg/dL	155.5 (138.5-177.6)	157.7 ± 29.0	162.4 (145.7-186.4)	178.7 ± 35.3
Triglycerides, mg/dL	112.7 (82.9-158.5)	129.3 (94.5-175.5)	119.0 (87.0-167.4)	126.5 (91.6-183.7)
HDL, mg/dL	47.4 ± 11.2	50.9 ± 11.6	49.9 (42.7-58.6)	50.0 (43.2-61.6)
Hypertension at baseline (no., %)	51 (9.9)	–	–	–
Hypertension in follow-up (no., %)	–	77 (18.7)	103 (28.9)	160 (53.9)

Normally distributed data are listed as means ± SD and non-normally distributed data are listed as median (interquartile range).
Abbreviations: BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HDL, high-density lipoprotein.

associations with BP responses to salt intake. Additionally, we found that these *CHRM3* SNPS were significantly associated with the development of hypertension and longitudinal BP changes over a 14-year period. These findings highlight the crucial role of the *CHRM3* gene in salt sensitivity and the long-term BP progression.

The present study found significant associations between several genetic variants (rs619288, rs58359377, rs10802811, rs6429147, rs373288072, rs114677844, and rs663148) within the *CHRM3* gene and BP responses to both low-salt and high-salt diets. These findings provide further evidence supporting the role of *CHRM3* gene mutations in salt sensitivity. An animal study conducted by Deng and associates[25] further confirmed this relationship. They identified a specific missense mutation (T1776C) in the intracellular domain of *CHRM3* through functional studies in the Dahl salt-sensitive (DSS) rat model. This mutation resulted in delayed internalization of M3R, increased cell surface receptors, prolonged functional signals, and ultimately, elevated BP. To further validate the role of enhanced M3R function on BP changes due to *CHRM3* mutation, the researchers generated a *CHRM3* gene-specific knockout DSS rat model and noted lower BP in *CHRM3*$^{-/-}$ rats compared to *CHRM3*$^{+/+}$ littermates.[25] In addition, Deng and associates[22] demonstrated that increased M3R signaling stimulated adrenal production of epinephrine, which subsequently influenced vascular function in the DSS model. However, there is no known polygenic mechanism that can explain why all hypertensive individuals would have the same *CHRM3*-T1667 missense mutation. This is evident as spontaneously hypertensive rats, despite being hypertensive, do not exhibit the *CHRM3*-T1667 mutation. In addition, the precise mechanisms behind the impaired vasodilation observed in *CHRM3*-knockout DSS rats due to delayed M3R internalization have yet to be fully elucidated. Therefore, further studies are required to unravel the mechanisms by which the identified risk loci contribute to the pathophysiology of salt sensitivity.

Acetylcholine plays a transmitter role during human heart development. From the 4th week after conception, a muscarinic response to acetylcholine can be detected. M3 muscarinic acetylcholine receptor has been reported to be associated with the regulation of BP and hypertension.[32,33] Razzaq and associates[34] found that pulegone prevented hypertension through activation of muscarinic receptors and cyclooxygenase pathway in L-NAME-induced hypertensive rats. Deng and associates [35] showed that eliminating M3R signaling reduced BP in DSS rats. As for other muscarinic receptor subtypes, M2 muscarinic receptor was found to be voltage sensitive, while altered sensitivity could result in clinical manifestations of disease states such as vagally-mediated atrial fibrillation and syndrome of inappropriate sinus tachycardia.[36] Researchers found that M5 muscarinic receptors trigger acetylcholine-induced Ca^{2+} signals and nitric oxide release in human brain microvascular endothelial cells.[37] Our study provides the first evidence of the associations between the SNPs rs10802764, rs12034970, rs6429147, rs58359377, and rs10802811 in the *CHRM3* gene and longitudinal changes in BP. Previous studies have shown that body mass index, physical activity, diet and smoking are risk factors for the development of hypertension.[38,39] We newly found that the *CHRM3* gene variant rs10802764 was associated with a decreased risk of hypertension after 14 years of follow-up (OR = 0.63, 95% CI: 0.43-0.92), while rs619288 was significantly associated with an increased risk of hypertension (OR = 1.74, 95% CI: 1.06-2.87). Our study provides direct evidence that *CHRM3* may be involved in the development of hypertension. It also suggests that *CHRM3* may have important theoretical significance and practical application in the prevention, individualized treatment and drug target finding of hypertension. However, no significant association was observed between rs619288 and BP changes during the 14 years of follow-up, probably because the incidence of hypertension and BP change are different phenotypes and the SNP loci studied were limited.

TABLE 6 The association between CHRM3 SNPs and BP changes during longitudinal follow-ups.

	BP (2004-2009)				BP (2004-2012)				BP (2004-2018)			
SNP	SBP change	DBP change	MAP change	PP change	SBP change	DBP change	MAP change	PP change	SBP change	DBP change	MAP change	PP change
rs10802764	0.192	0.897	0.466	0.103	0.048	0.396	0.122	0.022[b]	0.220	0.205	0.153	0.614
rs997148	0.703	0.718	0.971	0.348	0.783	0.734	0.944	0.614	0.266	0.559	0.366	0.370
rs12034970	0.704	**0.017**[b]	0.976	0.506	0.791	0.295	0.425	0.640	0.572	0.803	0.895	0.379
rs6429147	**0.027**	0.793	**0.033**[b]	**<0.001**	0.192	0.423	0.243	0.312	0.763	0.858	0.971	0.625
rs6692904	0.915	0.916	0.997	0.878	0.877	0.859	0.967	0.764	0.991	0.962	0.997	0.974
rs75804766	0.564	0.661	0.597	0.703	0.754	0.119	0.259	0.442	0.479	0.988	0.719	0.392
rs373288072	0.991	0.699	0.819	0.762	0.492	0.577	0.961	0.233	0.682	0.909	0.884	0.518
rs619288	0.363	0.620	0.464	0.361	0.181	0.490	0.267	0.265	0.960	0.206	0.440	0.384
rs58359377	0.569	0.163	0.283	0.813	0.700	**0.038**[a]	0.487	0.973	**0.017**	0.244	**0.045**[a]	**0.037**
rs114677844	0.297	0.685	0.433	0.269	0.632	0.934	0.871	0.435	0.281	0.279	0.230	0.514
rs663148	0.180	0.514	0.282	0.187	0.557	0.947	0.758	0.421	0.449	0.311	0.319	0.761
rs10802811	0.905	0.781	0.885	0.515	0.888	0.212	0.414	0.636	0.203	0.333	0.219	**0.044**[a]
rs4072234	0.853	0.861	0.837	0.929	0.681	0.482	0.522	0.977	0.980	0.080	0.284	0.163

Associations that were not significant under any model (additive, dominant or recessive) are presented as *p* values under additive model. BP change = BP at follow-up (2009, 2012 or 2018)—BP at baseline (2004).
Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.
Bold values emphasize that the *P*-values are less than 0.05.
[a]dominant model.
[b]recessive model.

TABLE 7 The association between *CHRM3* SNPs and hypertension incidence during longitudinal follow-ups.

	Incident hypertension (2004-2009)		Incident hypertension (2004-2012)		Incident hypertension (2004-2018)	
SNP	OR (95% CI)	*p*	OR (95% CI)	*p*	OR (95% CI)	*p*
rs10802764	0.85 (0.58-1.26)	.434	0.75 (0.52-1.09)	.137	0.63 (0.43-0.92)	.017
rs997148	0.96 (0.55-1.68)	.892	1.05 (0.61-1.73)	.874	1.26 (0.69-2.30)	.470
rs12034970	1.32 (0.69-2.51)	.408	0.78 (0.42-1.48)	.458	0.60 (0.29-1.21)	.151
rs6429147	1.73 (1.05-2.84)	.030	1.75 (1.07-2.88)	**.026**	1.36 (0.83-2.24)	.223
rs6692904	0.99 (0.63-1.56)	.977	1.22 (0.77-1.92)	.400	1.55 (0.94-2.54)	.083
rs75804766	1.21 (0.65-2.27)	.561	0.73 (0.39-1.37)	.328	0.67 (0.33-1.34)	.258
rs373288072	1.34 (0.90-2.00)	.155	1.04 (0.73-1.50)	.855	0.94 (0.65-1.36)	.754
rs619288	1.27 (0.78-2.07)	.349	1.08 (0.69-1.67)	.757	1.74 (1.06-2.87)	.029
rs58359377	1.07 (0.74-1.56)	.731	1.17 (0.81-1.69)	.405	1.11 (0.76-1.61)	.605
rs114677844	0.74 (0.30-1.81)	.517	0.46 (0.18-1.15)	.097	0.84 (0.39-1.81)	.673
rs663148	0.70 (0.29-1.73)	.451	0.43 (0.17-1.07)	.070	0.78 (0.36-1.68)	.541
rs10802811	0.92 (0.60-1.42)	.719	1.12 (0.76-1.65)	.593	0.95 (0.64-1.41)	.803
rs4072234	1.25 (0.83-1.88)	.297	1.16 (0.79-1.68)	.458	1.24 (0.84-1.85)	.287

Abbreviations: CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism.
Bold values emphasize that the *P*-values are less than 0.05.

The current study has some strengths. First, the participants were selected from nearby rural communities, the use of family pedigrees minimized potential confounding genetic associations arising from intra-individual and inter-individual differences in salt intake. In addition, the accurate measurement of 24-h urinary sodium excretion rates provided a reliable indicator of compliance with the prescribed dietary salt intake during each intervention period. Despite these strengths, several limitations of the study should be acknowledged. First, the study only examined a limited number of genotyped SNPs. Consequently, it is possible that less common genetic variants were not encompassed within the study, potentially impacting the comprehensiveness of the findings. Furthermore, we included sodium and potassium as confounders but ignored the effects of other nutritional behaviors on BP. Finally, generalization of the results may be limited since the study participants were exclusively obtained from northern China. To enhance the robustness and generalizability of the findings, validation studies should be conducted with ethnically diverse populations.

5 | CONCLUSIONS

In this study, we identified significant associations between genetic variants within the *CHRM3* gene and BP changes in response to salt intake. In addition, we observed strong associations between *CHRM3* genetic variants and the long-term progression of BP as well as the incidence of hypertension. These findings provide valuable insights into the genetic mechanisms involved in BP regulation and have the potential to contribute to clinical and public health applications.

AUTHOR CONTRIBUTIONS

Yang Wang and Jianjun Mu conceived and designed the experiments. Jianjun Mu was responsible for participant recruitment. Xi Zhang, Peng Bao, Mingfei Du, Guilin Hu, Chao Chu, Dan Wang, Chen Chen, Qiong Ma, Hao Jia, Yue Sun, Yu Yan, Yueyuan Liao, Zejiaxin Niu, Ziyue Man, Lan Wang, Weihua Gao, Hao Li, Jie Zhang, Wenjing Luo, and Xin Wang performed the experiments. Shi Yao and Xi Zhang analyzed the data. Xi Zhang and Yang Wang drafted the paper. Yang Wang and Jianjun Mu edited and revised manuscript. All authors read, critically revised and approved the final manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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