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Associations of genetic variations in NEDD4L with salt sensitivity, blood pressure changes and hypertension incidence in Chinese adults

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Abstract

Neural precursor cell expressed developmentally downregulated 4-like (NEDD4L), a member of the E3 ubiquitin-protein ligases, encoded by *NEDD4L* gene, was found to be involved in in salt sensitivity by regulating sodium reabsorption in salt-sensitive rats. The authors aimed to explore the associations of *NEDD4L* genetic variants with salt sensitivity, blood pressure (BP) changes and hypertension incidence in Chinese adults. Participants from 124 families in Northern China in the Baoji Salt-Sensitive Study Cohort in 2004, who received the chronic salt intake intervention, including a 7-day low-salt diet (3.0 g/day) and a 7-day high-salt diet (18 g/day), were ana-

Ze-Jiaxin Niu and Shi Yao have contributed equally to this work.

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lyzed. Besides, the development of hypertension over 14 years was evaluated. *NEDD4L* single nucleotide polymorphism (SNP) rs74408486 was shown to be significantly associated with systolic BP (SBP), diastolic BP (DBP) and mean arterial pressure (MAP) responses to low-salt diet, while SNPs rs292449 and rs2288775 were significantly associated with pulse pressure (PP) response to high-salt diet. In addition, SNP rs4149605, rs73450471, and rs482805 were significantly associated with the longitudinal changes in SBP, DBP, MAP, or PP at 14 years of follow-up. SNP rs292449 was significantly associated with hypertension incidence over the 14-year follow-up. Finally, this gene-based analysis found that *NEDD4L* was significantly associated with longitudinal BP changes and the incidence of hypertension over the 14-year follow-up. This study indicated that gene polymorphism in *NEDD4L* serve an important function in salt sensitivity, longitudinal BP change and development of hypertension in the Chinese population.

KEYWORDS
gene polymorphism, hypertension, NEDD4L, salt, salt sensitivity

1 | INTRODUCTION

Hypertension is an important risk factor for cardiovascular disease and all-cause mortality.[1,2] Previous studies have shown that hypertension is caused by both genetic and environmental factors and their interactions.[2,3] Among the environmental factors, high salt intake appears to be the major cause of increased blood pressure (BP). However, BP response varies among individuals after dietary salt intake, which was known as salt sensitivity.[4–6] Epidemiological data indicated that genetic predisposition may have a vital role in salt sensitivity.[7,8] Therefore, identifying genetic variants related to salt sensitivity would enhance our understanding of biological mechanisms of BP regulation and provide theoretical support for hypertension targeted therapy.[9,10]

Neural precursor cell expressed developmentally downregulated 4-like (NEDD4) is an E3 ubiquitin-protein ligase belonging to the HECT (homologous to E6-AP-carboxyl terminus) family and being involved in substrate recognition and ubiquitylation,[11–14] including NEDD4-1 and NEDD4-2(also named NEDD4L). NEDD4L is expressed in brain, heart, lung and kidney.[15,16] NEDD4L was shown to be involved in salt sensitivity of BP by regulating sodium homeostasis in mice. ENaC [epithelial Na(+) channel] play an important role in the regulation of sodium re-absorption. NEDD4L-mediated ubiquitylation of ENaC lead to its endocytosis and degradation, thus reducing sodium reabsorption.[17–23] Shi and colleagues[24] showed that the *NEDD4L* gene knockout mice had higher BP on a normal diet and a further increase in BP when on a high-salt diet. This association was also found in humans. An important evidence is Liddle syndrome, a type of hereditary hypertension, which is caused by human ENaC mutation. The mutated ENaC cannot be ubiquitinated by NEDD4L, resulting in an increase in its quantity and activity, which increases sodium re-absorption. However, the relationship between NEDD4L and salt intake and salt sensitivity of BP in humans have not been studied previously.

NEDD4L gene is located on chromosome 18q21 and consists of 41 exons, which contains >900 SNPs in the NCBI database. Recent studies have suggested that *NEDD4L* gene may be a candidate gene for hypertension. Luo and colleagues[25] demonstrated *NEDD4L* gene variants were involved in the development of essential hypertension, familial orthostatic hypertension and pulse pressure (PP). Besides, *NEDD4L* gene polymorphism affects BP levels in normal subjects. Dahlberg and colleagues[26] confirmed that the carriers of an intact NEDD4L C2-domain, encoded by the *NEDD4L* rs4149601 GG genotype, together with the C-allele of the *NEDD4L* rs2288774 polymorphism had higher BP. In addition, recent studies also found that an evolving new NEDD4L isoform (called isoform I), resulting from a frame-shift mutation, was associated with hypertensive phenotypes.[27] However, none of previous studies have fully considered the effect of gene-environment interactions on BP. Moreover, no study has yet examined whether genetic variants in the *NEDD4L* gene can predict long-term changes in BP or the development of hypertension over time.

Therefore, our objective is to prospectively analyze the relationship between genetic variants in *NEDD4L* gene and BP response to strict salt intervention, and explore the associations of *NEDD4L* genetic variants with longitudinal BP changes and the incidence of hypertension in our previously established Chinese cohort.

2 | METHODS

2.1 | Study participants

We established the Baoji salt sensitivity study cohort in 2004, which consisted of 514 adults from 124 families in seven villages of Baoji City, Shaanxi Province, China. A total of 334 non-parental subjects received a chronic salt intake intervention in 2004, and the detailed

study design has been published elsewhere.[28–34] Briefly, the first phase of the intervention was a 3-day baseline observation period, during which questionnaires and physical examinations were conducted. This was followed by a 7-day low-salt diet (3 g of NaCl or 51.3 mmol of sodium per day). Then, a high-salt diet (18 g of NaCl or 307.8 mmol of sodium per day) was administered for 7 days. Dietary potassium intake remained constant during the intervention phases.

To further identify the associations of potential genetic polymorphisms with longitudinal BP changes and hypertension incidence, we followed Baoji Salt-Sensitive Study cohort in 2009, 2012, and 2018, which was performed as previously described.[29–33] In the follow-up evaluations, a 3-day examination was performed as in 2004. BP value was measured three times a day, and the average of 9 BP values was taken for final analysis.

The ethics committee of First Affiliated Hospital of Xi'an Jiaotong University approved the study protocol (code: 2015–128). Written informed consents for the baseline assessment and for the intervention program were obtained from each participant. This study adheres to the principles of the Declaration of Helsinki. (ClinicalTrials.gov. registration number: NCT02734472).

2.2 | BP measurement and definition of BP response to dietary Intervention

BP was measured in the sitting position using a standard mercury sphygmomanometer as previously described.[34–37] BP was measured by trained and certified observers during the 3-day baseline observation period as well as on days 5, 6, and 7 of each of the three 7-day intervention periods. Hypertension was defined as systolic BP (SBP) of ≥140 mmHg, diastolic BP (DBP) ≥90 mmHg or as the use of antihypertensive drugs. The mean arterial pressure (MAP) was calculated as DBP + [1/3 × (SBP − DBP)]. PP was calculated as SBP − DBP.

BP changes from the low-salt intervention to the high-salt intervention may provide a more valid phenotype measure for salt-sensitivity because participants' salt intake was controlled during both phases. On the other hand, identifying genetic determinants of BP response to a low-salt diet from a usual diet should have more direct clinical and public health implications. Therefore, as described previously,[31–33] BP responses were defined as follows: BP response to the low-salt = BP on the low-salt diet—BP at baseline; and BP response to high-salt = BP on the high-salt diet—BP on the low-salt diet.

2.3 | Blood biochemical analyses

Fasting blood samples were obtained by peripheral venous puncture on the last day of each intervention period, and immediately centrifuged at 3000 × g for 10 min and stored at −80°C until analysis. Total cholesterol, triglycerides, high-density lipoprotein and fasting glucose levels were measured using an automatic biochemical analyzer (Hitachi, Tokyo, Japan). Details of these assays were described previously.[29–33]

2.4 | SNP selection and genotyping

Fourteen single nucleotide polymorphisms (SNPs) of *NEDD4L* gene (rs9956206, rs557036, rs563283, rs4940647, rs4149601, rs6566939, rs4149605, rs73450471, rs482805, rs74408486, rs292449, rs2288774, rs2288775, rs7228980) were selected, using the National Center for Biotechnology Information database (http://www.ncbi.nlm.nih.gov/projects/SNP) and the Genome Variation Server database (http://gvs.gs.washington.edu/GVS147/). The selection criteria for SNPs were as follows: the SNP frequency distribution of the tag site conforms to Hardy-Weinberg equilibrium (HWE) *P*-value ≥.05, the minor allele frequency (MAF) ≥.05, and the linkage disequilibrium coefficient $R^2 \geq .8$. All the genotyping experiments were performed by CapitalBio (CapitalBio Corp, Beijing, China) as previously described.[31–33]

2.5 | Statistical analyses

The quality control of parental SNP data, including genotyping call rate, Mendelian consistency, MAF and HWE, was performed using PLINK software (version 1.9, http://zzz.bwh.harvard.edu/plink/). We also used PLINK software to test the association of single marker with phenotypes. Three genetic models (additive, dominant and recessive) were also assumed for each SNP analysis by mixed-effect regression models. Models were adjusted for the fixed effects of baseline age, sex, and BMI, and the random effects of familial correlations. Bonferroni correction was used for adjustment of multiple testing.

For the analysis of the incidence of hypertension, 51 participants who had been diagnosed with hypertension at baseline were excluded. The association of each SNP with hypertension incidence was analyzed by generalized linear mixed models. Changes in age, sex, and BMI as fixed effects and familial correlation as a random effect were adjusted in the multivariable analysis by using *glmer* function in *lme4* R package.

Gene-based analysis was also performed to evaluate the overall association of a candidate gene with longitudinal BP changes over time and hypertension incidence.[31,32] The truncated product method (TPM), which combines *P*-values from single SNP association analyses, is an approach for evaluating the association between a candidate gene and a trait. Gene-based analysis was performed using *R* software (version 3.0.1; http://www.r-project.org).

3 | RESULTS

3.1 | Baseline characteristics and BP response to dietary intervention

Table 1 summarizes the baseline characteristics and BP responses to low-salt and high-salt diets in the original cohort of the Baoji Salt-Sensitive Study ($N = 514$). Baseline SBP and DBP were higher in probands compared to their siblings, spouses, and offspring. The changes of BP were consistent with salt intake. BP decreased in low-salt diet and increased in high-salt diet ($P < .05$).

TABLE 1 Baseline characteristics and BP response to chronic salt intake intervention

	Probands	Siblings	Spouses	Offspring	Parents
No. of participants	99	167	18	49	181
Age (years)	41.8 ± 8.4	39.8 ± 7.4	47.4 ± 6.1	23.3 ± 6.9	66.1 ± 8.3
Male (%)	69.7	49.1	26.3	49.0	48.4
Body mass index (kg/m^2)	23.0 ± 2.8	22.2 ± 2.9	23.1 ± 4.7	20.1 ± 2.7	20.4 ± 2.6
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	.22 ± 4.52	–
MAP change	4.71 ± 6.86*	3.22 ± 5.60	2.98 ± 5.61	.72 ± 3.79	–

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; SBP, systolic blood pressure.
*$P < .05$ versus the siblings, spouses or offspring.
**$P < .05$ versus the low-salt intervention.
***$P < .05$ versus the baseline levels.

As shown in Table S1, the urinary sodium excretion on the low-salt diet was significantly lower than at baseline, but significantly higher on the high-salt diet. The results showed that participants had good compliance to dietary intervention.

3.2 | *NEDD4L* and BP response to dietary intervention

Table 2 shows the genomic location, MAF, Hardy-Weinberg test and potential function prediction for each of the *NEDD4L* SNPs. None of the SNPs deviates significantly from HWE.

Table 3 presents the associations of the *NEDD4L* SNPs with BP response to dietary intervention. SNPs rs557036, rs563283, rs74408486, rs7228980 were significantly associated with DBP response to low-salt diet (β = -.310, P = .043 for rs557036; β = -.624, P = .034 for rs563283; β = .310, P = .015 for rs74408486; β = -.319, P = .024 for rs7228980), among which SNP rs74408486 was also significantly associated with SBP and MAP response to low-salt intervention (β = .275, P = .035). In addition, SNPs rs292449, rs2288775 were significantly associated with PP response to high-salt diet (β = -.280, P = .015 for rs292449; β = .222, P = .048 for rs2288775).

3.3 | Characteristics of the participants in the longitudinal follow-up cohort study

Table 4 shows the demographics and BP of the participants at baseline (2004) and at follow-up (2009, 2012, and 2018). Over 14 years of follow-up, the mean SBP, DBP, and MAP increased by 21.2, 7.9, and 12.3 mmHg, respectively. In addition, 160(53.9%) subjects developed hypertension.

3.4 | Association analyses for longitudinal BP changes and incidence of hypertension

Table 5 presents the associations of individual SNPs in *NEDD4L* gene with the 5-year (2004–2009), 8-year (2004–2012), and 14-year (2004–2018) BP changes. *NEDD4L* SNP rs9956206 and rs73450471

TABLE 2 Information on genotyped SNPs of NEDD4L gene

SNP	Position	Region	Alleles[a]	MAF	*P*-value*,[b]	Potential function prediction
rs9956206	55732315	Intronic	T/G	.1621	.02719	—
rs557036	55784264	Intronic	G/A	.3949	.219	DHS、TFBS
rs563283	55794327	Intronic	G/T	.2139	.8145	DHS
rs4940647	55808797	Intronic	A/G	.1662	.7898	DHS
rs4149601	55816791	Exonic	A/G	.1537	.3801	—
rs6566939	55829768	Intronic	G/A	.4294	.1669	DHS、TFBS
rs4149605	55861273	ncRNA_exonic	C/A	.3844	.3195	DHS、TFBS
rs73450471	55876665	Intronic	T/C	.4106	.5406	DHS、TFBS
rs482805	55881103	Intronic	C/T	.497	.651	DHS、TFBS
rs74408486	55882989	Intronic	G/T	.08815	.3662	—
rs292449	55895081	UTR5	G/C	.2078	.8225	—
rs2288774	55983330	Intronic	C/T	.3943	.112	DHS、TFBS
rs2288775	55983364	Intronic	G/A	.2913	.1392	TFBS
rs7228980	55985449	Intronic	T/A	.07831	1	—

Abbreviations: DHS, DNase I hypersensitive site; MAF, minor allele frequency; SNP, single nucleotide polymorphism; TFBS, transcription factor binding site.
[a]Alleles are presented as major: minor allele.
[b]Parents only (parental generation).
**P*-values of Hardy-Weinberg equilibrium test.

TABLE 3 Characteristics of the study participants at baseline and during follow-ups

Characteristics	Baseline in 2004	Follow-up in 2009	Follow-up in 2012	Follow-up in 2018
Sex (M/F)	267/247	208/204	185/171	155/142
Age (years)	48.6 ± 19.8	53.3 ± 14.2	56.6 ± 19.0	62.3 ± 12.1
Body mass index (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 (n, %)	51 (9.9)	–	–	–
Hypertension incidence (n, %)[a]	–	77 (18.9)	103 (28.9)	160 (53.9)

Notes: Non-normally distributed variables are expressed as the median (interquartile range).
All other values are expressed as mean ± SD or n, %.
Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein; MAP, mean arterial pressure; SBP, systolic blood pressure.
[a]Participants with hypertension at baseline were excluded.

were significantly associated with the longitudinal changes in SBP, DBP, MAP, or PP at 5 years of follow-up. SNP rs9956206, rs2288774, and rs2288775 were significantly associated with the longitudinal changes in SBP, DBP, MAP, or PP at 8 years of follow-up. SNP rs4149605, rs73450471, and rs482805 were significantly associated with the longitudinal changes in SBP, DBP, MAP, or PP after 14 years of follow-up after Bonferroni correction. In addition, gene-based analysis found that *NEDD4L* was significantly associated with longitudinal DBP change (P_{TPM} = .042) and MAP change (P_{TPM} = .029) over a 14-year follow-up.

TABLE 4 Characteristics of the study participants at baseline and during follow-ups

Characteristics	Baseline in 2004	Follow-up in 2009	Follow-up in 2012	Follow-up in 2018
Sex (M/F)	267/247	208/204	185/171	155/142
Age (years)	48.6 ± 19.8	53.3 ± 14.2	56.6 ± 19.0	62.3 ± 12.1
Body mass index (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 (n, %)	51 (9.9)	–	–	–
Hypertension incidence (n, %)[a]	–	77 (18.9)	103 (28.9)	160 (53.9)

Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein; MAP, mean arterial pressure; SBP, systolic blood pressure.
Notes: Non-normally distributed variables are expressed as the median (interquartile range).
All other values are expressed as mean ± SD or n, %.
[a]Participants with hypertension at baseline were excluded.

TABLE 5 Association of NEDD4L SNPs with blood pressure changes from baseline to the follow-ups in the longitudinal follow-up cohort study

SNP	BP(2004-2009)				BP(2004-2012)				BP(2004-2018)			
	SBP change	DBP change	MAP change	PP change	SBP change	DBP change	MAP change	PP change	SBP change	DBP change	MAP change	PP Change
rs9956206	**.03**	**.04**	**.02**	.17	**.01**	**.00**	**.00**	.40	**.04**	.69	.22	**.02**
rs557036	.72	.78	.73	.79	.87	.82	.83	.97	.16	.60	.31	.15
rs563283	.57	.58	.97	.22	.18	.16	.13	.50	.78	.10	.25	.33
rs4940647	.58	.69	.97	.27	.36	.72	.50	.33	.83	.87	.84	.88
rs4149601	.53	.33	.38	.93	.87	.41	.57	.68	.84	.82	.96	.66
rs6566939	.72	.61	.90	.37	.43	.47	.41	.62	.47	.47	.47	.87
rs4149605	.85	.39	.68	.34	.36	.38	.33	.60	.20	.23	.92	**.00**[a]
rs73450471	.73	**.04**[a]	.49	.10	.33	.07	.12	.92	.12	.68	.31	**.03**[b]
rs482805	.98	.14	.38	.25	.23	.06	.08	.88	.31	.43	.99	**.03**[a]
rs74408486	.70	.69	.97	.39	.69	.97	.85	.57	.70	.22	.35	.61
rs292449	.86	.11	.31	.31	.32	.09	.13	.99	.91	.90	.98	.80
rs2288774	.62	.46	.50	.92	.07	.69	.24	**.03**	.38	.13	.17	.93
rs2288775	.72	.46	.54	.93	**.01**[b]	.35	**.02**[b]	**.01**[b]	.17	.11	.10	.61
rs7228980	.84	.95	.95	.74	.80	.58	.84	.44	.83	.55	.80	.44

Abbreviations: BP, blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; PP, pulse pressure; SBP, systolic blood pressure; SNP, single nucleotide polymorphism.
Note: For associations that were not significant under any model, *P* values for an additive model are listed. All genetic models are based on the minor allele of each SNP.
[a]Dominant model.
[b]Recessive model.

Finally, we analyzed the relationships of *NEDD4L* SNPs with 5-year (2004–2009), 8-year (2004–2012), and 14-year (2004–2018) incidences of hypertension, which are shown in Table 6. SNP rs292449 was significantly associated with hypertension incidence after 14-year follow-up [*OR* (95%CI) = .55 (.10–1.03), *P* = .02]. Furthermore, *NEDD4L* was found to be significantly associated with hypertension incidence from gene-based analysis over 14-year follow-up (P_{TPM} = .0104) after adjustment for multiple testing.

TABLE 6 Association of individual SNPs with hypertension incidence in the longitudinal follow-up cohort study

SNP	Incident hypertension (2004-2009)		Incident hypertension (2004-2012)		Incident hypertension (2004-2018)	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
rs9956206	.01 (−.54 to .52)	.98	−.11 (−.59 to .35)	.64	.19 (−.29 to .68)	.44
rs557036	.02 (−.38 to .41)	.94	−.26 (−.63 to .08)	.14	.31 (−.06 to .69)	.10
rs563283	.23 (−.25 to .69)	.34	−.08 (−.52 to .34)	.71	.05 (−.37 to .49)	.79
rs4940647	−.12 (−.66 to .40)	.66	−.21 (−.69 to .25)	.37	−.04 (−.54 to .45)	.86
rs4149601	−.30 (−.26 to .84)	.29	.07 (−.42 to .55)	.77	.24 (−.24 to .74)	.32
rs6566939	.25 (−.63 to .12)	.19	.12 (−.19 to .45)	.43	−.23 (−.57 to .09)	.17
rs4149605	.07 (−.32 to .46)	.74	.29 (−.05 to .64)	.10	−.28 (−.64 to .08)	.13
rs73450471	−.14 (−.55 to .25)	.48	−.28 (−.65 to .06)	.11	.13 (−.21 to .50)	.44
rs482805	.01 (−.36 to .38)	.96	−.33 (−.67 to −.00)	.05	−.21 (−.57 to .14)	.23
rs74408486	.53 (−.25 to .60)	.10	−.25 (−.91 to .36)	.43	.21 (−.40 to .85)	.50
rs292449	.13 (−.35 to .51)	.57	−.05 (−.49 to .37)	.80	.55 (.10 to 1.03)	.02
rs2288774	−.30 (−.68 to .07)	.12	−.11 (−.45 to .20)	.48	.19 (−.14 to .54)	.27
rs2288775	−.17 (−.59 to 23)	.41	−.23 (−.60 to .13)	.21	.11 (−.25 to .49)	.53
rs7228980	−.49 (−1.30 to .20)	.19	.30 (−.26 to .88)	.28	.37 (−.24 to 1.01)	.24

Abbreviations: CI, confidence interval; OR: odds ratio; SNP: single nucleotide polymorphism.

4 | DISCUSSION

In this study, we found that *NEDD4L* genetic variations are involved in salt sensitivity of BP after strict salt intervention, and identified several new SNPs in Chinese population. These findings confirm the potentially important contributions of *NEDD4L* gene to salt sensitivity and long-term BP regulation. In addition, this study also enhanced our understanding of the genetic factors in salt sensitivity of BP.

Previous animal studies have indicated that NEDD4L is involved in salt sensitivity by regulating Na^+ homeostasis. ENaC [epithelial Na(+)channel] plays an important role in the sodium reabsorption. Staub and colleagues[38] suggested that NEDD4L binds to the PY motifs of ENaC subunits via its WW domains, ubiquitinates them, and decreases their expression on the apical membrane in a mouse model of Liddle's syndrome. Shi and colleagues[24] showed that *NEDD4L* knockout mice had higher expression levels of all three ENaC subunits in kidney, and had higher BP on a high-salt diet compared with wild type mice. Minegishi and colleagues[19] established NEDD4L C2 domain knockout mice model and confirmed that loss of NEDD4L C2 isoform induced to salt-sensitive hypertension under high-salt diet in vivo. In addition, limited data indirectly suggest the association between *NEDD4L* genetic variations and salt sensitivity in human. Jonas and colleagues[26] also found that carriers of the rs4149601 GG-genotype together with the rs2288774 CC-genotype had significantly higher salt sensitivity after 8-week salt intervention in Swedish normotensive subjects. Our study is the first study to investigate the association of *NEDD4L* gene with salt sensitivity of BP in Chinese. We found that SNPs rs557036, rs563283, rs74408486, rs7228980 were significantly associated with BP responses to low-salt diet while SNPs rs292449, rs2288775 were significantly associated with BP responses to high-salt diet in Chinese cohort. Our study also found some new *NEDD4L* SNPs involved in salt sensitivity. However, the mechanism of how the relevant SNPs contribute to salt sensitivity of BP at the molecular and cellular levels remains unclear and deserves further investigation.

This is also the first study exploring the association of *NEDD4L* gene variants with longitudinal BP and the occurrence of hypertension in a Chinese cohort. We found that SNP rs4149605, rs73450471 and rs482805 were significantly associated with the longitudinal changes after 14 years of follow-up. In addition, SNP rs292449 was significantly associated with hypertension incidence over 14 years. Our study provides direct evidence that *NEDD4L* gene may be involved in long-term BP regulation and hypertension, although its genetic and physiological mechanisms remain unclear. This relationship was also reported in other populations. Kouremenos and colleagues[39] showed that *NEDD4L* SNPs (rs513563 and rs3865418) were associated with hypertension in white Americans and two other SNPs (rs4149589 and rs3865418) were related to Greek whites among 128 families (including Greek Caucasians, African-Americans and Caucasian-Americans) with a history of hypertension. Fava and colleagues[40] found that carriers of the rs4149601 GG genotype had higher DBP and faster progression of DBP over time and carriers of the rs2288774 C-allele had higher SBP in 118 Caucasian families from Sweden. In addition, the genetic variation of *NEDD4L* gene may reduce the NEDD4L activity in vivo, leading to hypertension. Dahlberg and colleagues[41] demonstrated that a *NEDD4L* genotype combination of the rs4149601GG and rs2288774 CC was correlates with lower NEDD4L activity, higher cross-sectional BP and higher CVD incidence in Sweden (MalmoÍDiet and Cancer study). Limited evidence indirectly suggests that *NEDD4L* genetic variations involved in long-term BP in humans, however, our study was based on a long-term follow-up cohort and provided evidence that NEDD4L genetic variations affected long-term BP and the development of hypertension. Therefore, this will help us to further

understand the mechanism of hypertension from a genetic point of view and provide new ideas for targeted therapies of hypertension.

This study has several strengths. First, we conducted a family pedigree-based cohort study in Chinese Han population. The lifestyle and eating habits of participants from homogeneous environment were basically the same. Bias caused by population stratification is unlikely to occur. Second, because of strictly controlled salt intake in our study, confounding genetic associations due to differences in dietary salt intake within individuals (daily) and between individuals were reduced. The 24-hour urinary sodium and potassium excretion at each stage showed good compliance with dietary interventions. Finally, strict quality control procedures were used for genotyping and data collection. Measurement error was reduced because an average of nine individual BP measurements were taken at baseline and at each follow-up. However, this study also has some limitations. This study lacked a validation cohort; therefore, the new findings need to be replicated in other cohorts with different genetic backgrounds. In addition, because of the limited number of genotype SNPs in *NEDD4L* gene, less common genetic variations may be ignored in this study.

In conclusion, our study reported for the first time that *NEDD4L* gene polymorphisms are significantly associated with BP response after salt intervention, longitudinal BP changes, and incident hypertension in a Chinese cohort. Findings of the current study provide evidence for potential prevention and a possible therapeutic target for hypertension in the future. Furthermore, this work contributes to the cumulative understanding of the genomic mechanisms that regulate BP and the development of hypertension.

AUTHOR CONTRIBUTIONS

Yang Wang and Jian-Jun Mu conceived and designed the experiments. Jian-Jun Mu was responsible for subject recruitment. Yang Wang, Ze-Jiaxin Niu, Xi Zhang, Ming-Fei Du, Ting Zou, Chao Chu, Yue-Yuan Liao, Gui-Lin Hu, Chao Chu, Dan Wang, Qiong Ma, Yu Yan, Hao Jia, Ke-Ke Wang, Yue Sun, Zi-Yue Man, Lan Wang, Wei-Hua Gao, Hao Li, Yong-Xing Wu, Chun-Hua Li, Ke Gao, and Jie Zhang performed the experiments. Shi Yao and Ze-Jiaxin Niu analyzed the data. Ze-Jiaxin Niu and Yang Wang drafted the paper. Tie-Lin Yang and Jian-Jun Mu edited and revised manuscript. All authors read, critically revised and approved the final manuscript.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

PERMISSION TO REPRODUCE MATERIAL FROM OTHER SOURCES

Not applicable.

CLINICAL TRIAL REGISTRATION

ClinicalTrials.gov. registration number: NCT02734472.

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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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