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Original Article

Genetic variation and association analyses of *NEDD4* gene in Kazak Chinese patients with hypertension

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Abstract: To analyze the association between the genetic variations of neural precursor cell expressed developmentally down-regulated 4 gene (*NEDD4*) and hypertension in Kazakh Chinese. The sequences of *NEDD4* gene exons were sequenced in 96 Kazakh Chinese with hypertension to identify representative variations. A case-control study was conducted by genotyping the representative variations in 287 Kazakh hypertensives and 411 normotensives. Replication population was 343 Uygur hypertensives and 724 normotensives. All subjects were selected from population-based cross-sectional studies of metabolic disease. Thirteen novel and 15 known single nucleotide polymorphism (SNPs) or mutations, including 6 missense mutations, were identified. Of the four representative SNPs genotyped, only rs2303580 was association with hypertension in Kazakh (additive *P*/*Pc*=0.020/0.160) without Bonferroni's correction. The result was replicated in Uygur (additive/dominant *P*=0.089/0.028, *Pc*=0.174/0.056). By adjusting for age and BMI, the observed association would no longer be statistically significant in Kazakh (additive OR (95%CI) 1.035(0.802-1.336), but remained statistically significant in Uygur (additive/dominant ORs (95%CI) 1.323 (1.069-1.637), 1.521(1.146-2.020)). The rs2303580 genotypes were not association with blood pressure levels in Kazakh. Although by multiple linear regression analysis and by applying Bonferroni's correction, the genotypes were significant association with diastolic blood pressure levels (AA>AG>GG) in Uygur normotensive controls (*P*/*Pc*=0.003/0.018), the direction of difference was not in accordance with the association between the qualitative hypertension phenotype and the genotype shown (G risk allele). Our data indicates that the association between the *NEDD4* genetic polymorphisms and hypertension phenotype should be replicated in further studies using larger and racially diverse populations.

Keywords: Genetic epidemiology, genetic association study, hypertension, ethnic

Introduction

The precise control of blood pressure occurs via Na+ homeostasis and involves the precise regulation of the epithelial Na+ channel (ENaC) in the aldosterone-sensitive distal nephron. This has been corroborated by the linkage of mutations in the genes encoding ENaC subunits and Liddle's syndrome, a heritable form of human hypertension [1]. Mapping of these mutations on ENaC indicated that inactivation of PY motifs is responsible and leads to the proposition that the channel interacts via its PY motifs with the WW domains of the Nedd4/Nedd4-like (neural precursor cell expressed developmentally down-regulated 4) ubiquitin protein ligase family[2,3]. It is now well established that the cell surface expression of ENaC is controlled via ubiquitylation by this protein family [4,5]. Several studies have proposed that mutations in NEDD4L may be responsible for these blood pressure phenotypes [6-10]. It is therefore reasonable to assume that naturally occurring polymorphic genetic variations of *NEDD4* being on chromosome 15q21.3 of ubiquitin-mediated degradation of ENaC might underlie partly the variation seen between individuals in their susceptibility to hypertension and the progression of their disease. And chromosome 15q may be a quantitative trait locus for blood pressure [11,12]. The present study first evaluated the association between the *NEDD4* genetic polymorphisms and hypertension phenotype by performing variation screening of the *NEDD4* gene exons

and then carrying out a genetic association study in Kazakh Chinese, a relatively isolated population [10] with high prevalence of hypertension [13].

Materials and methods

Subjects and DNA samples

The sample size for Kazakh hypertension subjects (SBP ≥150mmHg or DBP ≥95mmHg) was 287 (70 patients with medication treatment of hypertension) (male: female ratio 140:147, age 48.56±8.93 years, SBP 157.61±20.83mmHg, DBP 101.34±10.42 mmHg), whereas that for Kazakh normotensive controls (SBP≤130mmHg and DBP≤85mmHg) was 411 (male: female ratio 166:245, age 42.45±8.01 years, SBP 114.61±8.84 mmHg, DBP 76.11±6.25mmHg). The sample size for replication study in Uygur Chinese comprised 343 hypertensives (91 patients with medication treatment of hypertension) (male:female ratio 128:215, age 54.52±9.17 years, SBP 166.03±19.16 mmHg, DBP 96.26±14.05 mmHg) and 724 normotensives (male :female ratio 258:466, age 48.36±11.13 years, SBP 112.12±11.32 mmHg, DBP 69.42±8.61 mmHg) (**Table 1**). The Kazakh and Uygur Chinese study subjects were selected respectively from the population-based cross-sectional study of obesity, hypertension, diabetes, dyslipidemia during January to March 2008 and 2007 among Kazakh and Uygur population, both of relatively isolated population with a relatively homogeneous environment [10, 14]. Subjects with secondary hypertension (estimated by history, examination, and laboratory evaluation), excessive drinking, cancer and use of contraceptives were excluded from this study. The criteria of hypertensive case was systolic blood pressure (SBP) ≥150mmHg or diastolic blood pressure (DBP) ≥95mmHg or anti-hypertension treatment in ordering to increase the positive proportion, and the criteria of normotensive control was SBP≤130mmHg and DBP≤85mmHg and no history of any anti-hypertensive medications in ordering to decrease the false negative proportion. The characteristics of the subjects analyzed in the present study are summarized in **Table 1**. Genomic DNA was prepared from the blood sample of each subject by using the PAXgene blood DNA Kit (A QIAGEN/BD Company). Written consents were obtained from all subjects before any data collection and measurements. This study was approved by the Ethnic Committee of the People's Hospital of Xinjiang Uygur Autonomous Region.

Diagnostic criteria and Measurements

Overnight fasting blood samples were taken in the morning from the antecubital vein. Samples were divided into aliquots, separated within 30 min and stored at −80°C until transport to People's hospital of XinJiang Uygur Autonomous Region–certified laboratories for analyses. The blood pressure measurement was performed by trained and certified observers three times per subject at least 10 min of rest in a sitting position. Weight, height and waist circumference (WC) were measured using standard techniques with the participants in light clothing and barefoot. Body mass index (BMI) was calculated as weight (kg)/height $(m)^2$. The measurements were taken twice in the continuous two days, and the mean of the two weight, height, WC values and six blood pressure values was used for further calculations. In addition to performing routine blood examination that included lipid profiles, glucose levels, blood/urine electrolyte, and anthropometric measurements, a set of questionnaires were also completed, including demographic information, personal history, detailed previous history, family history of diseases and lifestyle, et al.

Variation screening and Genotyping representative variations of NEDD4 Gene

All exons, exon-intron boundaries and the putative promoter region, including the 5'- and 3'-untranslated regions (UTRs) (~1kb) of *NEDD4* gene were sequenced from genomic DNA isolated from 96 unrelated hypertension individuals (including 48 males and 48 females) using an ABI 3130xl genetic analyzer (BigDye Terminator Cycle sequencing V3.1/V1.1 Kit; Applied Biosystems, Foster City, California, USA). The nucleotide sequence (Gen-Bank accession ID NT_010194.16) was used as a reference sequence. Primer specifics and optimized PCR conditions are available upon request.

After considering their function and linkage disequilibrium (LD) (a r^2 cutoff of 0.8) among the identified genetic variations, the genotypes of 4 common single nucleotide polymorphisms (SNPs) with a minor allele frequency (MAF) of greater than 10% were determined by the

TaqMan- PCR system. The ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Warrington, UK) was used for end-point detection and allele calling. Negative and positive controls were included in each plate. For genotyping quality control, the case and control subjects were distributed randomly across the plates, and the samples sequenced also were genotyped for detecting genotyping errors. The call rate for genotyping was 98.9% and the discrepancy in the concordance of duplicates was <0.2%. The primers and probes for the TaqMan-PCR method (supplement) were chosen based on the information available on the Applied Biosystems Inc. website (http://myscience. appliedbiosystems.com). For the novel SNP, 77943A>C (N407H), the primers were 5'-GGTGATTGTAAA CCAGAAATGTCAGAAAT-3' (Foward) and 5'-GCAG ATGTCCTATGCATGAGCTTAA-3' (Reverse), and the probes were VIC- TCTGAATCAGAATTAAGCT (for the A allele) and FAM-CTGAATCAGAATGAAGCT (for the C allele).

Statistical analysis

Quantitative data were expressed as means ±Std. or median whether they were normal distribution. Simple comparisons of the clinical data between case and control groups were analyzed by *t*-test or student's *t*-test. Significant skewed variables were detected by Histogram for the continuous traits. Subsequently, triglycerides (TG), low density lipoprotein-cholesterol (LDL-c), fasting glucose (FBG), 2-hour postprandial glucose (2HPG) were normalized by log-transformation before statistical comparisons, and all P-values were derived from analyses of transformed data. The minor allele was too rare for most polymorphisms to give enough power. Therefore, additive and dominant models were used to detect the allelic association. In the additive model, χ^2 test was performed according to Sladek et al [15]. In the dominant model, frequencies of the homozygous genotype for the major allele were compared using a 2 ×2 contingency table. A test of independence was performed using Pearson's χ2 method. The odds ratio (OR) and 95% confidence interval (CI) were calculated by logistic regression. We coded genotypes as 0, 1, and 2, depending on the number of copies of the minor allele. OR adjusted for age and BMI was calculated using logistic regression with genotypes, age, and BMI as independent variables. Effect size of one allele for SBP and DBP levels was analyzed by a multiple linear regression after controlling age, BMI, medication treatment and disease status, where appropriate. Data analyses were performed by the SPSS15.0 statistics package. For all analyses, *P* values <0.05 were considered statistically significantly. *P* values 0.05-0.10 were considered statistically borderline significantly. *Pc* values were calculated for multiple testing using Bonferroni's inequality method and defined as *P* values (single test) × number of tests. Hardy–Weinberg equilibrium (HWE), LD was performed using the program SNPAlyze, version 7.0 Pro (DYNACOM Co. Ltd., Mobara, Japan).

Results

Identification of genetic variations of NEDD4 gene in Kazakh patients with hypertension

We performed systematic variation screening of the *NEDD4* gene in 96 Kazakh hypertension patients (male: female ratio 48:48) and identified 28 variations, including 4 variations in 3'-untranslated region (UTR), 14 variations in intron regions, 6 missense mutations of the 10 variations in exon region. Among the identified 6 missense mutations, 3 novel variants, which are not found in NCBI SNP-database, are 77291T>G (S189R), 77748 C>T (R342W) with MAF <2%, and 77943A>C (N407H) with MAF 27.0%. After considering their function (missense mutation), LD and MAF, four common SNPs, 77943A>C (N407H), rs2303580 (132882A>G, R607Q), rs8028559 (154845T>C), and rs11550869 (165622G>C) were selected as representative for genotyping experiments in 698 Kazakh Chinese (**Table 2**).

Study subjects and replication population

The clinical characteristics of Kazakh study subjects and Uygur replication population are shown in **Table 1**. The case–control cohort used in this investigation was matched for ethnicity, culture and geographical locations. All variables were significant differences between hypertensive cases and normotensive controls (all *P* <0.05), excluding high density lipoprotein-cholesterol (HDL-c) (*P*=0.061 in Kazakh, *P*=0.138 in Uygur).

Relationship of the genetic variations of NEDD4 gene with hypertension risk and blood pressure quantitative traits

Table 1. Comparison of clinical characteristics in hypertensives(SBP≤130mmHg and DBP≤85mmHg) and normotensive (SBP≥150mmHg or DBP≥95mmHg) subjects

	Kazak(698)				Uygur(1067)			
	Normotensive	Hypertensives	statistic values	*P*- values	Normotensive	Hypertensives	statistic values	*P*- values
n (men/women)	411(166/245)	287(140/147)			724(258/466)	343（128/215）		
Age (years)	42.45±8.01	48.56±8.93	-9.265	<0.001	48.36±11.13	54.52±9.17	-9.539	<0.001
BMI(Kg/m2)	25.08±3.48	28.85±4.71	-11.370	<0.001	26.10±4.25	28.40±4.15	-8.073	<0.001
WC(cm)	80.13±10.44	90.34±12.92	-10.923	<0.001	82.53±10.64	88.93±11.29	-9.004	<0.001
SBP (mmHg)	114.61±8.84	157.61±20.83	-32.965	<0.001	112.12±11.32	166.03±19.16	-48.272	<0.001
DBP (mmHg)	76.11±6.25	101.34±10.42	-36.664	<0.001	69.42±8.61	96.26±14.05	-32.597	<0.001
TC(mmol/l)	4.89±1.05	5.20±1.04	-3.760	<0.001	4.31±1.16	4.69±1.19	-5.051	<0.001
HDL-c (mmol/l)	1.47±0.39	1.42±0.38	1.889	0.059	1.08±0.33	1.12±0.32	-1.486	0.138
LDL-c (mmol/l)[a]	2.89	3.18	-4.422	<0.001	2.24	2.71	-5.212	<0.001
TG(mmol/l)[a]	0.84	1.05	-6.843	<0.001	1.19	1.50	-7.058	<0.001
FBG (mmol/l)[a]	4.78	5.02	-4.471	<0.001	5.15	5.37	-2.868	0.004
2HPG(mmol/l)[a]	6.00	6.48	-3.589	<0.001	6.44	7.13	-2.425	0.016

Abbreviations: BMI, body mass index; WC, waist circumference; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; HDL-c, high density lipoprotein- cholesterol; LDL-c, low density lipoprotein-cholesterol; TG, triglyceride; FBG, fasting blood glucose; 2HPG, 2 hour postprandial glucose.

[a]Log-transformed traits; Values of log-transformed variables are presented as median and other un-transformed variables are presented as mean ± std.

P values were analyzed using *t*-test or student's *t*-test.

Table 2. Identified polymorphisms of NEDD4 Gene function region in the 96 Kazakh with hypertension

SNPs Allele1> Allele2	LD	Amino acid chang	Region	Allele 1 Homo(n)	Hetero (n)	Allele 2 Homo(n)	Total	Minor allele frequency	Flanking sequence	dbSNP ID
26945 T>A			intron1	23	40	23	86	0.50	ttgatatatattttt[t/a]aaaaaattgtgtctt	rs6493829
26969 T>C			intron1	49	31	6	86	0.262	gtgtcttagttaact[t/c]ataagtacatgaaaa	
27095 T>A			Intron2	59	21	6	86	0.192	ctaggtaagtatatc[t/a]atttggattataatt	
76402 G>A			Intron5	92	1	0	93	0.005	tataattgaaacaag[g/a]ttggctgtgtttgag	
76821 A>G	a	M33V	exon6	82	3	0	85	0.018	GATAGCCATGTTCAC[A/G]TGTGCTTCAAAAGAC	rs1912403
77291 T>G		S189R	exon6	83	1	1	85	0.018	TGTCATCAGTGACAG[T/G]AGTAGTTATACTTTT	
77576 G>A		L284L	exon6	82	3	0	85	0.018	TCTCCAACAAGTCT[G/A]TGTACTCTTCTGAGC	
77748 C>T		R342W	exon6	86	1	0	87	0.006	GAAGTAAGAGACATA[C/T]GGCCGCTTCACAGG	
77771 G>A		S349S	exon6	59	22	6	87	0.195	TCACAGGAAGGGCTC[G/A]TTACAGAAGAAAATT	rs7174459
77943 A>C *	*b*	*N407H*	exon6	48	31	8	87	0.270	TCAGAAATTAAGCTT[A/C]ATTCTGATTCAGAGT	
78221 A>G		L499L	exon6	87	2		89	0.011	TAAAGTGGATAATTT[A/G]TCAAGAGACAGCAAC	
119511G>A	b		intron6	48	35	8	91	0.280	ctaaaggaattgagc[g/a]tattactaattatat	
123925C>T			intron9	90	1	0	91	0.011	AAATGACTTATTTAC[C/T]TAAAACCAGTGGCTC	
***132882G>A** *	***c***	***R607Q***	exon12	10	44	34	88	0.636	AAAGTGTTGACAACC[G/A]AGAGTCTTCCGAGgt	***rs2303580***
133025A>G	c	N626S	exon13	10	44	34	88	0.636	CCACCATGTATAGCA[A/G]CCAGGCCTTCCCATC	rs2303579
144759A>T	d		intron16	43	36	7	86	0.291	TTTACCTgtaagtgt[a/t]tagaaatgctaaccc	rs12232351
***154845T>C** *	***d***		intron22	48	34	7	89	0.270	taaaggatgtccatc[t/c]gacctctcatcttt	*rs8028559*
155062A>G	e		intron23	73	15	2	90	0.106	Aggtttgtgaattttg[a/g]tcagttaaaatggca	
155065A>T	a		intron23	87	3	0	90	0.017	ttgtgaattttgatc[a/t]gttaaaatggcaca	rs12906245
155121A>G	e		intron23	73	15	2	90	0.106	ttaatacgtacactt[a/g]atacattcctaggct	
159692A>G	a		intron27	87	2	0	89	0.011	ccttctgggtctgta[a/g]ctattactaggtaag	rs12908466
160547C>T		Y1189Y	exon28	91	2	0	93	0.011	ATTTGCTGAACTATA[C/T]Ggtaaggattttcca	
160572A>T	d		intron28	52	34	7	93	0.258	tttccatagatcatt[a/t]aaaaaatggaataat	rs8026172
160619G>T	d		intron28	52	34	7	93	0.258	catggctgacgaatcc[g/t]caagcttccatcatt	rs8024944
164400T>G	a	3'-UTR		82	3	0	85	0.018	AAAGTATTAAAGCCT[T/G]TCTCTTGCCTGCATA	rs12899701
164420A>G		3'-UTR		53	33	0	86	0.192	TTGCCTGCATATCCT[A/G]TTGACCATTGGTATA	rs7162435
***165622C>G** *	***e***	*3'-UTR*		69	14	2	85	0.106	ACCCTCATTGTCATG[C/G]CAGATTGTCAGAAGT	***rs11550869***
166177G>A	b	3'-UTR		43	34	8	85	0.294	TCCCACTAGGGGCTC[G/A]TGGTCTGGAAGAAAC	rs3088077

The apparent linkage disequilibrium (LD), defined by r-square more than 0.8, was indicated by a-d in the LD column. * These SNPs were used for genotyping analysis. The A of the initiator Met codon is denoted nucleotide +1. The genome sequence retrieved from GenBank (accession ID: NT_010194.16, GI:37540936) was used as a reference sequence.

Table 3. Association between the *NEDD4* genetic polymorphisms and Hypertension

	SNP type	Geno-types	Normotensive n(%) (SBP≤130mmHg and DBP≤85mmHg)	hypertensives n(%) (SBP≥150mmHg or DBP≥95mmHg)	χ^2- *values* (Additive/ dominant)	*P-values* (Additive/ Dominant)	OR (95%CI) (Additive/ dominant)
Kazakh	154845T/C	TT	194(47.8)	137(47.9)	1.956/0.001	0.376/0.975	0.915(0.690-1.215)/
	rs8028559	TC	175(43.1)	131(45.8)			0.936(0.654-1.338)
		CC	37(9.1)	18(6.3)			
Kazakh	165622G/C	GG	315(78.2)	223(79.1)	0.537/0.082	0.764/0.774	0.882(0.582-1.335)/
	rs11550869	GC	81(20.1)	56(19.9)			0.876(0.560-1.372)
		CC	7(1.7)	3(1.1)			
Kazakh	77943A/C	AA	208(50.6)	138(48.4)	1.743/0.322	0.418/0.570	1.007(0.771-1.315)/
	novel	AC	155(37.7)	120(42.1)			1.055(0.738-1.508)
		CC	48(11.7)	27(9.5)			
Kazakh	132882A/G	AA	156(38.1)	92(32.2)	7.791/2.617	0.020/0.106	1.035(0.802-1.336)/
	rs2303580	AG	174(42.5)	152(53.1)			1.253(0.863-1.818)
		GG	79(19.3)	42(14.7)			
Uygur	132882A/G	AA	358(49.4)	145(42.3)	4.846/4.806	0.089/0.028	1.323(1.069-1.637)/
	rs2303580	AG	297(41.0)	162(47.2)			1.521(1.146-2.020)
		GG	69(9.5)	36(10.5)			

Data are n (%). In the additive model, odds ratios (ORs) were expressed per difference in number of minor alleles. In the dominant model, ORs were shown as heterozygotes and minor allele homozygotes compared with major allele homozygotes. OR for each SNP was adjusted simultaneously for age and BMI.

Table 4. Comparison of blood pressure levels among different genotypes of rs2303580 polymorphism

	Genotypes	Kazakh (n=698, control 411/case 287) SBP(mmHg)	Kazakh (n=698, control 411/case 287) DBP(mmHg)	Uygur (n=1067, control 724/case 343) SBP(mmHg)	Uygur (n=1067, control 724/case 343) DBP(mmHg)
Controls	AA	114.79±9.29	76.16±6.29	112.83±11.03	70.36±8.43
	AG	114.56±8.61	76.42±6.24	111.47±11.43	68.68±8.61
	GG	113.68±9.07	75.03±6.49	111.02±12.18	67.59±9.04
	Additive P-values	0.789	0.624	0.224	***0.003 B=-1.390***
Cases	AA	155.06±20.74	100.68±8.11	169.26±19.08	97.43±13.50
	AG	159.68±20.45	102.26±11.27	165.03±19.09	95.21±14.96
	GG	156.06±22.11	99.72±12.21	157.51±17.03	96.26±11.77
	Additive P-values	0.739	0.468	***0.064***	0.452
Combined	AA	130.25±24.54	85.57±13.84	129.13±29.09	78.18±15.93
	AG	135.20±27.16	88.24±15.66	130.38±29.48	78.04±16.96
	GG	128.66±25.24	83.76±14.81	126.96±26.20	77.42±16.94
	AdditiveP-values	0.668	0.519	***0.047 B=- 0.3516***	***0.210***

Abbreviations: SBP, systolic blood pressure, DBP, diastolic blood pressure.

Data are means ± std from multivariate ANOVA with age, BMI as covariates.

P-values were derived from multiple linear regression analysis using the additive model after adjusting for age, BMI, medication treatment and disease status, where appropriate.

Genotypes distribution of the four common SNPs in Kazakh case and control were in HWE ($P>0.05$) (**Table 3**). Of these four SNPs genotyped, only the SNP rs2303580 (132882A>G, R607Q) was significant association with hypertension in Kazakh Chinese without applying Bonferroni's correction (additive *P*=0.020) (**Table 3**), and the result was replicated in 1067 Uygur Chinese (additive/dominant *P*=0.089/0.028, *Pc*=0.178/0.056). By adjusting for age and BMI, the observed association with hypertension phenotype for the SNP rs2303580 (132882A>G, R607Q) would no longer be statistically significant in Kazakh (additive OR (95%CI) 1.035(0.802-1.336), but remained statistically significant in Uygur (ORs (95%CI) 1.323(1.069-1.637), 1.521(1.146-2.020), respectively under additive and dominant models).

The association of the SNP rs2303580 (132882A>G, R607Q) with blood pressure quantitative traits was also analyzed by multiple linear regression controlling age, BMI, medication treatment and disease status, where appropriate. (**Table 4**). The rs2303580 (132882A>G, R607Q) genotypes were not significant association with SBP and DBP levels in Kazakh Chinese. By applying Bonferroni's method and adjusting for confounding factors, the genotypes were statistically significant association with DBP levels (AA>AG>GG) in Uygur normotensive controls (*P/Pc*=0.003/0.018), and were not association with SBP levels in Uygur combined samples (*P/Pc*=0.047/0.282).

Discussion

This study is one of serial researches about susceptibility to hypertension in Kazakh population [10]. The research strategy was selected for the following reasons: a) HapMap project does not provide genetic information for Kazakh Chinese, so Tag-SNPs of *NEDD4* gene could not be specific for Kazakh in this study. b) Sequencing exons have high sensitivity for identification rare and common variants compared with genome-wide sequencing, and the strategy may be extendable to diseases with more complex genetics through larger sample sizes and appropriate weighting of non-synonymous variants by predicted functional impact [16].

In the present study, we identified 28 SNPs, including 13 novel variations, in the *NEDD4* gene. MAF of *NEDD4* genetic polymorphisms, rs2303580, rs8028559 and rs11550869, in Kazakh Chinese (40.2%, 30.8%, 10.8%, respectively) was different from in European (21.7%, 36.0%, 9.2%, respectively) and Han Chinese (44.4%, 35.6%, 10.0%, respectively). Of these four representative SNPs genotyped, only the SNP rs2303580 (132882A>G, R607Q) was statistically significant association (additive *P*=0.020) with hypertension phenotype in Kazakh. By applying Bonferroni's correction, the observed association with hypertension phenotype would no longer be statistically significant for the SNP rs2303580 (132882A>G, R607Q). Moreover, no association of this variant with quantitative measures of blood pressure in Kazakh sample suggests that the association may be false..The result has been replicated in Uygur population. By applying Bonferroni's correction, there was statistically borderline significant association (dominant *P/Pc* =0.028/0.056) with hypertension phenotype for the SNP rs2303580 (132882A>G, R607Q) in Uygur Chinese. By adjusting for age and BMI factors, the observed association with hypertension phenotype for the SNP rs2303580 (132882A>G, R607Q) remained statistically significant in Uygur (ORs (95%CI) 1.323(1.069-1.637), 1.521(1.146-2.020)), respectively under additive and dominant models). Our results in the multiple linear regression analysis revealed a significant association of this SNP with DBP levels (*P*=0.003) in Uygur normotensive controls and SBP levels (*P*=0.047) in Uygur combined samples (cases + controls) under an additive genetic model. However, although the P-value associated with DBP levels (AA>AG>GG) remained statistically significant after applying Bonferroni's correction in Uygur normotensive controls (*Pc*=0.018), the directions of difference were not in accordance with the association between the qualitative hypertension phenotype and the genotype shown (G risk allele). From these results, it appears that the association of *NEDD4* genetic polymorphisms with hypertension still needs to be replicated in another population.

Many factors may contribute to variable results of genetic association study, major ones being sample size and ethnic stratification. The major advantage of the study is that the subjects were selected from the population-based cross-section studies. Kazakh which dwells north of Xinjiang and Uygur which dwells South of Xinjiang are a relatively isolated population with a

relatively homogeneous environment [10,14]. However, the sample size of this study may not have been sufficiently powerful to detect modest effects of the *NEDD4* genetic polymorphisms, and the case-control cohort was not matched for age and gender. Although the association of the SNP rs2303580 (132882A>G, R607Q) with hypertension phenotype in Kazakh Chinese was replicated in Uygur Chinese, the results may be a false positive findings for population stratification.

Up to date, there are no reports about the association study between *NEDD4* genetic variations and metabolic diseases. We first reported the distribution of *NEDD4* genetic variations in Kazakh hypertension patients and found that the SNP rs2303580 (132882A>G, R607Q) may be associated with hypertension phenotype. Further studies should replicate this finding using larger and racially diverse populations.

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