GMR Genetics and Molecular Research
Online Journal
ISSN:1676-5680

Correlation between natriuretic peptide receptor C (*NPR3*) gene polymorphisms and hypertension in the Dai people of China

H. Sun, Z.Q. Yang, S.Y. Liu, L. Yu, K. Huang, K.Q. Lin, J.Y. Chu and X.Q. Huang

The Department of Medical Genetics, Institute of Medical Biology, Chinese Academy of Medical Sciences and Peking Union Medical College, Kunming, China

Corresponding author: X.Q. Huang
E-mail: huangxiaoqin@imbcams.com.cn

Genet. Mol. Res. 14 (3): 8786-8795 (2015)
Received November 4, 2014
Accepted March 23, 2015
Published August 3, 2015
DOI http://dx.doi.org/10.4238/2015.August.3.2

ABSTRACT. Hypertension affects one-fifth of the world population. Genome-wide association studies (GWAS) have identified several single nucleotide polymorphisms (SNPs) that correlated with hypertension in large samples. However, the genetic mutations leading to hypertension might differ among various populations, as they have different origins and are subjected to different environmental pressures. Therefore, additional studies are urgently needed to verify the GWAS findings across different populations. This study focused on the natriuretic peptide receptor C gene (*NPR3*), one of the hypertension-positive genes identified in a GWAS of an East Asian population. The correlation analysis between *NPR3* and hypertension was replicated in 450 Chinese Dai (235 patients *vs* 215 controls) and 484 Chinese Mongolian (211 patients *vs* 273 controls) individuals. The positive SNP identified by GWAS analysis and three other tag SNPs representing the *NPR3* linkage disequilibrium (LD) block regions were selected for genotyp-

ing. The results revealed that the rs1173766 polymorphism was associated with the occurrence of hypertension (χ^2 = 6.87, P = 0.0088), and that the T allele should be protective in the Dai ethnic group. Considering a close LD block at the 3' end of the *NPR3* gene in the East Asian population, we speculate that there might be a mutation in the last five exons or the 3' untranslated region of *NPR3* that could change the structure or expression of the *NPR3* gene. However, in the Mongolian ethnic group, these SNPs were not associated with the incidence of hypertension, suggesting population heterogeneity for the genetic factors that contribute to hypertension.

Key words: Population diversity; Hypertension; GWAS; Genetics; NPR3

INTRODUCTION

Hypertension is one of the major diseases affecting the health of 20-30% of the world population, and primary hypertension accounts for approximately 95% of all hypertension cases (Kearney et al., 2005). Hypertension might occur due to the combined effects of environmental and genetic factors, and genetic factors have always been considered important causes of primary hypertension. It has been shown that the heritability of primary hypertension is approximately 60% in twins (Hunt et al., 1989), and other studies have demonstrated the heritability of hypertension to be as high as 30-50% (Hunt et al., 1989).

The genetic etiology of hypertension has been investigated since the 1980s. More than 200 genes have been shown to be related to the occurrence of hypertension, and several important mutations in the genes regulating blood pressure have also been identified (Kunes and Zicha, 2009). However, these investigations do not fully explain the genetic etiology of hypertension because of the complexity of the genetic factors that contribute to hypertension and the underlying population heterogeneity. As technology has advanced, genome-wide association studies (GWASs) have gradually been applied to the analysis of complex diseases. A number of research institutes have conducted GWAS analyses of hypertension and have investigated the genetic etiology of hypertension at the level of whole genome (Xu and Yan, 2013). For example, the *NPR3* gene encoding the natriuretic peptide receptor protein C has been demonstrated to be associated with the occurrence of hypertension in both European (International Consortium for Blood Pressure Genome-Wide Association Studies et al., 2011) and Asian populations (Kato et al., 2011) based on meta-analyses of GWASs. Although the *NPR3* gene was reported to have an association with hypertension in these two meta-analyses, the positive correlations are not observed in all ethnic groups. This suggests that, for complex diseases such as hypertension, populations with different origins and geographical environments might have their own characteristic pathogenic loci for hypertension. Therefore, it is crucial to verify the results from these GWASs across different populations.

The present study investigated the positive single nucleotide polymorphism (SNP) rs1173766 identified in the previous GWAS for hypertension as well as three loci located in the *NPR3* gene to verify the findings of the GWAS. Genotyping analysis was performed for the *NPR3* gene in 235 Dai patients with hypertension and in 215 healthy Dai individuals, as well as in 211 Mongolian patients with hypertension and in 273 healthy Mongolian individuals. We aimed to explore the correlation between polymorphisms in these loci and hypertension.

MATERIAL AND METHODS

Study subjects

The study was approved by the Ethics Committee at the Chinese Academy of Medical Sciences and Peking Union Medical College, and signed informed consents from all study subjects were obtained. Patients with hypertension and normal controls of the Mongolian and Dai ethnic groups were respectively collected from the pastoral area of Damaoqi, Inner Mongolia, and the Xishuangbanna Dai autonomous prefecture, Yunnan. The enrolled patients presented no secondary signs of hypertension, such as proteinuria. None of the patients had any history of myocardial infarction. Healthy individuals with normal blood pressures and normal electrocardiographs were recruited as normal controls. The three generations traced backward from each of the patients with hypertension or healthy control individuals were of the same ethnic group as the proband (i.e., the parents and the maternal and paternal grandparents of each subject in this study). Blood pressure was measured by the internationally standardized method. Three continuous measurements of blood pressure were performed for each subject, and the average of the three measurements was taken as the blood pressure of the subject. Phase I hypertension as defined by the World Health Organization (1999) (i.e., systolic blood pressure greater than 140 mmHg or diastolic blood pressure greater than 90 mmHg) served as the diagnostic criterion for selection of the patients with hypertension. Height, weight, age, and gender were recorded for all subjects. In total, 211 specimens of peripheral blood from the Mongolian patients with hypertension and 273 specimens of peripheral blood from the corresponding healthy controls were qualified after screening. Furthermore, 235 specimens of peripheral blood from the Dai patients with hypertension and 215 specimens of peripheral blood from the corresponding healthy controls were also qualified after screening.

Selection and genotyping of tag SNPs

Centered on the SNP rs1173766, which is near the 3' end of the *NPR3* gene and is positively associated with hypertension in the East Asian population based on GWAS, three SNPs in the direction of the *NPR3* gene were selected for investigation. According to the haplotype analysis of the Beijing Han and Japanese populations (CHB+JPT) in the HapMap Project (International HapMap Consortium et al., 2007), *NPR3* is covered by three linkage disequilibrium (LD) blocks. The rs1173766 SNP is located in the LD block at the 3' portion of the gene. For the other two LD blocks near the central and the 5' portions of the gene, we respectively selected rs10061804 and rs2302954 as the tag SNPs. To increase the genetic variability of the SNP markers in the LD block that contained rs1173766, rs1173756, also in the same LD block, was selected for study; this SNP is located in the 3' untranslated region of the gene. The locations of the four SNPs within *NPR3* are shown in Figure 1A. Analysis of the LD blocks was performed using the Haploview software (Barrett et al., 2005) based on the HapMap data of the CHB+JPT population, and the results are displayed in Figure 1B.

After collecting 250 μL peripheral blood, genomic DNA from leukocytes was extracted using an AxyPrep Blood Genomic DNA Miniprep Kit (Axygen, Hangzhou City, China). SNP genotyping was conducted using the single base extension SNaPshot method. The fragments containing the four SNPs described above were amplified by the polymerase chain reaction (PCR); the primer sequences are listed in Table 1. The PCR reagents were purchased from

TaKaRa Co., Ltd. (Dalian, China). The amplification reagents were as follows: 20 ng genomic DNA, 1.2 μL 25 mM $MgCl_2$, 1.6 μL 2.5 mM dNTPs, 1 μL 5 μM forward primer, 1 μL 5 μM reverse primer, 0.16 μL 55 U/μL Taq polymerase, 2.5 μL 10X PCR buffer, and an appropriate amount of water to reach a total volume of 20 μL. The amplification conditions consisted of 30 cycles of the series 94°C for 15 s, 60°C for 30 s, and 72°C for 15 s, followed by 72°C for 10 min.

The PCR products were purified, and the residual primers and dNTPs were removed by exonuclease *Exo*I treatment (Fermentas, Waltham, MA, USA) and FastAP thermosensitive phosphatase (Fermentas). The reaction system included 3 μL PCR product, 0.2 μL 20 U/μL *Exo*I, 0.8 μL 1 U/μL FastAP, 0.7 μL *Exo*I Buffer, and an appropriate amount of water to obtain a total volume of 7 μL. The reaction conditions were 37°C for 15 min, followed by 80°C for 15 min.

The purified products were subjected to single nucleotide sequencing reactions for the corresponding locus, according to the instructions of the SNaPshot® Multiplex Kit (Life Technologies, Carlsbad, CA, USA). Electrophoresis was performed on an ABI3130 instrument (Life Technologies) and the results were analyzed using the GeneMapper software (Life Technologies).

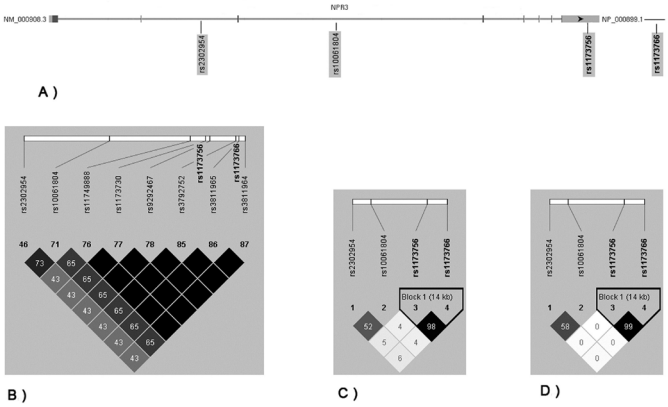

Figure 1. Locations of the four SNPs at the *NPR3* locus and their haplotype configurations. **A.** Diagram showing the positions of the four SNPs in the *NPR3* gene. **B.** Haplotype configuration diagram and matching r^2 values based on HapMap data for Japanese and Han Chinese populations. **C. D.** Haplotype configuration diagrams and matching r^2 values for the Dai and the Mongolian ethnic groups utilized in this study. The values shown in these subfigures are all LD r^2. SNP, single nucleotide polymorphism; LD, linkage disequilibrium.

Table 1. Primer sequences for genotyping the four SNPs using SNaPshot.

SNP		Primer sequences (5'-3')
rs2302954	Forward	CTG TGC TCT TAG CTT TGG TGA C
	Reverse	TGA GGA CTA GAG ACC GTG CTG G
	Typing	TTT TTT TTT TTT TTT TTT TCC CCC AAA GTG TCT GTG CTC
rs10061804	Forward	TAC CAC CTG TAG AGC TGG AAG
	Reverse	TGG TTC AGC CAA GAA GAA GCA
	Typing	TTT TTT TTT TTT TTT AGA TAA AAG GAT AAA TGC AG
rs1173756	Forward	TTA CTC AAG GCA CAT GTG CCT T
	Reverse	CAA ATA GCC AGT GTA TAC TGG
	Typing	TGT AAG ACA TGC AGT CAA CAA
rs1173766	Forward	TCA GAG CAG GTA CAT TCT CAT C
	Reverse	CCC TTC CAT GCT GTG GAA GCT
	Typing	TTT TTT CTT CTG GTG GAT TCA TGG TCT

SNP = single nucleotide polymorphism.

Statistical analysis

After the genotypes of the loci were determined, the data from the patients with hypertension were compared with those of the other subjects. The quantitative data are reported as the means ± standard deviation. The statistical differences among the quantitative data were tested using the Mann-Whitney *U* test, while the differences among the qualitative data and gene frequencies were determined via the chi-square test. The power of the chi-square test was estimated by using the power and sample size calculation software PS 3.0 (Dupont and Plummer, 1998). The relationships between the different genotypes and hypertension were also compared using the chi-square test and were divided into recessive and dominant modes for discussion. In addition, multi-factor logistic regression was applied to test the genotypes and other factors that might affect hypertension. After correction for the related factors, the association of each genetic polymorphism with the occurrence of hypertension was determined, and the odds ratio (OR) was estimated. The allele frequency and genotype statistics were computed using the PLINK software (Purcell et al., 2007), and Hardy-Weinberg equilibrium (HWE) tests were also performed using the same software. Other statistical analyses were performed using the SPSS17 software (SPSS, Chicago, IL, USA). The LD values between the loci were calculated using Haploview, and the LD map was plotted.

RESULTS

General subject information

The characteristics of the subjects are listed in Table 2. For all 450 Dai and 484 Mongolian individuals, the average values of the gender, age, body mass index (BMI), and blood pressure were calculated, and the statistical differences were compared. The gender data were analyzed with the chi-square test, and other measurement data were compared using the Mann-Whitney *U* test. For the two ethnic groups in this study, no significant difference was detected in the gender ratio between the patient and control groups. However, the age and BMI were significantly different between groups. This result suggests that hypertension is affected by a variety of factors.

Table 2. Survey indicators and statistical analysis of the study participants.

	Dai ethnic group			Mongolian ethnic group		
	Hypertensive	Normal	P value	Hypertensive	Normal	P value
Number of people	235	215		211	273	
Gender (male/Female)	79/156	59/156	0.156	92/119	127/146	0.522
Age (years)	59.07 ± 11.22	49.67 ± 12.18	<0.001*	52.20 ± 9.27	49.51 ± 10.17	0.003*
BMI (kg/m^2)	22.59 ± 3.55	21.32 ± 3.01	<0.001*	26.11 ± 5.52	24.51 ± 9.81	<0.001*
Systolic blood pressure (mmHg)	165.58 ± 21.90	123.08 ± 13.00	<0.001*	146.10 ± 14.43	116.41 ± 11.62	<0.001*
Diastolic blood pressure (mmHg)	93.80 ± 13.18	73.93 ± 10.03	<0.001*	98.21 ± 9.58	79.64 ± 6.32	<0.001*

Quantitative data are reported as means ± standard deviation and were tested with the Mann-Whitney *U* statistical method. Qualitative data were analyzed by the chi-square test. *P < 0.05. BMI = body mass index.

Allelic and genotypic frequency comparisons

All of the samples were successfully genotyped using the SNaPshot method. The allele frequencies at the four polymorphisms were analyzed by the chi-square test (Table 3) for the patient and control groups. The genotype frequencies of these four SNPs are listed in Table 4. All four polymorphisms were consistent with HWE in the patient and control groups at a significance level of $\alpha = 0.05$. After Bonferroni's correction, rs1173766 was found to be related to hypertension only in the Dai ethnic group ($\chi^2 = 6.87$, $P = 0.0088$). According to the result from the PS 3.0 software analysis, the power of this chi-square test was 0.739, when the test was performed using 235 patients and 215 controls. Furthermore, in the Dai ethnic group, although the P value of rs1173756 ($\chi^2 = 5.71$, $P = 0.0169$) was less than 0.05 in the initial analysis, the statistical significance of the difference disappeared after Bonferroni's correction. The power of this chi-square test was 0.658.

Table 3. Comparison of the *NPR3* SNP frequencies between hypertensive and normal populations.

Locus	Dai ethnic group			Mongolian ethnic group		
	Hypertensive (Frequency) N = 235	Normal (Frequency) N = 215	P value	Hypertensive (Frequency) N = 211	Normal (Frequency) N = 273	P value
rs1173766						
C	370 (0.787)	306 (0.712)		266 (0.630)	322 (0.590)	
T	100 (0.213)	124 (0.288)	0.0088*	156 (0.370)	224 (0.410)	0.1997
rs1173756						
C	370 (0.787)	309 (0.719)		264 (0.626)	322 (0.590)	
T	100 (0.213)	121 (0.281)	0.0169	158 (0.374)	224 (0.410)	0.2578
rs10061804						
G	372 (0.791)	341 (0.793)		300 (0.711)	365 (0.668)	
A	98 (0.209)	89 (0.207)	0.9548	122 (0.289)	181 (0.332)	0.1583
rs2302954						
T	383 (0.815)	338 (0.793)		322 (0.763)	393 (0.720)	
A	87 (0.185)	92 (0.214)	0.2788	100 (0.237)	153 (0.280)	0.1288

The chi-square test was performed for the four loci in each ethnic group. The data of each ethnic group were considered to comprise a data set. *In accordance with the principle of Bonferroni's correction, $P < 0.0125$ (0.05/4) was defined as statistically significant. SNP = single nucleotide polymorphism.

Table 4. Comparison of the genotype frequencies of the four *NPR3* SNPs between the hypertensive and normal populations.

Locus	Dai ethnic group			Mongolian ethnic group		
	Hypertensive (Frequency) N = 235	Normal (Frequency) N = 215	P value of chi-square test	Hypertensive (Frequency) N = 211	Normal (Frequency) N = 273	P value of chi-square test
rs1173766						
CC	144 (0.613)	104 (0.484)		82 (0.389)	95 (0.348)	
CT	82 (0.349)	98 (0.456)		102 (0.483)	132 (0.484)	0.400
TT	9 (0.038)	13 (0.060)	0.021*	27 (0.128)	46 (0.168)	
rs1173756						
CC	144 (0.613)	105 (0.488)		80 (0.379)	95 (0.348)	
CT	82 (0.349)	99 (0.460)		104 (0.493)	132 (0.484)	
TT	9 (0.038)	11 (0.051)	0.030*	27 (0.128)	46 (0.168)	0.441
rs10061804						
GG	147 (0.626)	133 (0.619)		113 (0.536)	122 (0.447)	
GA	78 (0.332)	75 (0.349)		74 (0.351)	121 (0.443)	
AA	10 (0.043)	7 (0.033)	0.819	24 (0.114)	30 (0.110)	0.107
rs2302954						
TT	155 (0.660)	131 (0.609)		126 (0.597)	144 (0.527)	
TA	73 (0.311)	76 (0.353)		70 (0.332)	105 (0.385)	
AA	7 (0.030)	8 (0.037)	0.534	15 (0.071)	24 (0.088)	0.305

*$P < 0.05$; SNP = single nucleotide polymorphism.

Because the allele frequency distribution of rs1173766 is associated with the occurrence of hypertension, we also performed the chi-square test for the genotype distribution for this SNP under different genetic models (Table 5). With the assumption that the low frequency T allele is the risk allele, the TT genotype was found to be associated with the incidence of hypertension in the recessive mode of inheritance. In the dominant mode, both genotypes containing the T allele (that is, TT and CT) were found to be related to hypertension. The chi-square test revealed that a statistically significant difference was detectable only in the dominant model for the Dai ethnic group, with a P value of 0.006 ($\chi^2 = 7.56$). The OR of the TT and CT genotypes between the patients with hypertension and the normal controls was 0.750 [95% confidence interval (CI): 0.610-0.922]. This finding also suggested that the TT and CT genotypes in the Dai ethnic group were protective factors for hypertension and might be associated with blood pressure reduction.

Table 5. Genotype distributions of rs1173766 in the Mongolian and Dai ethnic groups.

rs1173766	Dai ethnic group			Mongolian ethnic group		
	Hypertensive (Frequency) N = 235	Normal (Frequency) N = 215	P value /OR (95%CI)	Hypertensive (Frequency) N = 211	Normal (Frequency) N = 273	P value /OR (95%CI)
Recessive mode						
TT	9 (0.038)	13 (0.060)	0.276	27 (0.128)	46 (0.168)	0.217
CC+CT	226 (0.962)	202 (0.940)	/-	184 (0.872)	227 (0.832)	/-
Dominant mode						
TT+CT	91 (0.387)	111 (0.516)	0.006*	109 (0.517)	141 (0.516)	0.998
CC	144 (0.613)	104 (0.484)	/0.750 (0.610-0.922)	102 (0.483)	132 (0.484)	/-

For each ethnic group, the chi-square test was performed for two genotypic groups. The data of each ethnic group were considered to comprise a data set. The chi-square test revealed no significant differences, and the odds ratio (OR) values were not calculated. *In accordance with the principle of Bonferroni's correction, $P < 0.025$ (0.05/2) was defined as statistically significant. CI = confidence interval.

Haplotype analysis and logistic regression analysis

The LD values (r^2) among the four selected SNP loci were calculated using the Haploview software (Barrett et al., 2005) (Figure 1C and D). In the Dai and Mongolian ethnic groups, the linkage relationships of the four SNPs were consistent with the predictions of HapMap based on the CHB+JPT data. In the Dai and Mongolian ethnic groups, rs1173756 and rs1173766 were found to be closely linked ($r^2 > 0.95$), and were not located in the same LD block region as the other two tag SNPs. Therefore, to increase the genetic variability of the markers, the haplotype arrangement of rs1173756 and rs1173766 was further analyzed. However, the results revealed that, in the Dai ethnic group, the CC and TT haplotypes accounted for 75.1 and 24.6% respectively, and the frequencies of haplotypes CT and TC, which could increase the polymorphism of the analyzed locus, were only 0.3 and 0%, respectively. Thus, the analysis of the haplotype arrangement did not increase the locus polymorphism. Additional statistical tests for the patients and controls yielded results similar to those obtained with a single locus (data not shown).

Hypertension is caused by multiple factors. Therefore, we performed a binary logistic regression analysis for the correlations of the genotype, age, and BMI with hypertension to investigate comprehensively the relationships between the occurrence of hypertension and its various influencing factors. The results are presented in Table 6, and revealed that age (OR: 1.070; 95%CI: 1.050-1.089; $P < 0.001$) and BMI (OR: 1.121; 95%CI: 1.053-1.194; $P < 0.001$)

remained risk factors for hypertension even after correction, and that the TT+CT genotype was likewise statistically positive after correction for age and BMI (OR: 0.659; 95%CI: 0.473-0.992; P = 0.046). This result suggested that the TT or CT genotypes in the Dai ethnic group might have a correlation with the effects of age and BMI on the occurrence of hypertension that is protective against elevated blood pressure.

Table 6. Odds ratio (OR) and 95% confidence interval (CI) of the OR calculated for the TT+CT genotype and the hypertension risk factors by multivariate logistic regression.

Risk factor	Dai ethnic group		
	OR	95%CI	P
TT+CT genotype	0.659	0.437-0.992	0.046*
Age (year)	1.070	1.050-1.089	<0.001*
BMI (kg/m^2)	1.121	1.053-1.194	<0.001*

*P < 0.05; BMI = body mass index.

DISCUSSION

Kato et al. (2011) performed a meta-analysis of the GWAS results from East Asian populations and proposed four new genetic polymorphisms associated with an increase in blood pressure, including rs1173766 in the *NPR3* gene. This meta-analysis focused primarily on certain populations in East Asia, including the Japanese, Korean, and Malaysian groups, as well as some of the Han ethnic groups in eastern China. China is a populous country with many (56) ethnic groups. The correlation between the *NPR3* gene and hypertension in ethnic groups other than the Han is also worthy of investigation. Certain mutations leading to hypertension might have a higher frequency in specific ethnic groups and could be the main genetic factor leading to the rise of blood pressure in these ethnic groups. Therefore, it remains necessary to re-verify the GWAS findings in populations with different genetic backgrounds.

In the present study, to verify the GWAS findings, we respectively selected two ethnic groups that had different genetic backgrounds from the Han people (Sun et al., 2013). One was the Dai people who live in southern China, and the other is the Mongolian ethnic group, originating in Northern China. Based upon our results, the rs1173766 polymorphism in the *NPR3* gene was found to be related to hypertension in the Dai ethnic group. The C allele, which is present at a high frequency in this population, might be the respective risk allele for hypertension in this ethnic group, while the low frequency T allele might serve as a protective factor. This result agrees with the findings of the GWAS meta-analysis in East Asian populations (Kato et al., 2011). However, in the Mongolian population, an association between the occurrence of hypertension and rs1173766 was not found.

The *NPR3* gene encodes the natriuretic peptide receptor C, which, unlike the other two natriuretic peptide receptors, might be a non-guanylyl cyclase receptor. Similar to the other natriuretic peptide receptors, natriuretic peptide receptor C is known to regulate blood pressure and the volume of the extracellular liquid through interaction with natriuretic peptides (Anand-Srivastava, 2005). Therefore, this gene might be functionally related to the occurrence of hypertension. To expand our analysis, we selected three SNPs located in the *NPR3* gene surrounding rs1173766. These three SNPs were respectively located in several different LD blocks of the *NPR3* gene. However, our analysis revealed that, with the exception of the finding that rs1173766 was statistically associated with hypertension in the Dai ethnic group,

an association between the *NPR3* gene and hypertension was not indicated for any of the other loci. According to the JPT+CHB HapMap data, there is a region with close LD at the 3' end of *NPR3*, covering the last five exons of *NPR3* with a length of approximately 100 kb. The rs1173766 SNP is located in this region, and its statistically positive result suggests that mutations changing the structure or expression of the *NPR3* gene might exist in the last five exons of *NPR3* as well. It has been reported that a 37-amino-acid cytoplasmic domain at the end of *NPR3* is sufficient to reproduce the function of this receptor in inhibiting the cyclase activity of adenylate cyclase. (Pagano and Anand-Srivastava, 2001; Anand-Srivastava, 2005). Therefore, the last exon of *NPR3* that encodes these 37 amino acids might deserve further study.

In the present study, the relationships between the *NPR3* polymorphisms and the occurrence of hypertension were not consistent between the two ethnic groups, which might have been due to the genetic heterogeneity of the populations. The frequency of the statistically positive rs1173766 polymorphism revealed a significant difference between the Dai and the Mongolian populations; the percentages of the T allele were 28.8 and 41.0% in the Dai and Mongolian healthy control groups, respectively ($\chi^2 = 15.58$, $P < 0.0001$). Considering that the T allele might represent a protective factor, we speculated that the putative *NPR3* mutation that causes hypertension might have a higher frequency in the Dai than in the Mongolian ethnic group. Natriuretic peptide increases serum sodium levels and reduces sodium re-absorption in the kidney (Widmaier et al., 2008). As its receptor, *NPR3* is, therefore, also closely related to the utilization of sodium. Thus, genetic mutations causing hypertension likely differ between the Dai and Mongolian peoples, who have different genetic backgrounds.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported in part by the Peking Union Medical College Youth Fund & the Fundamental Research Funds for the Central Universities (#3332013085 and #33320140013). We also received support from the Chinese National High Technology Research and Development Program (#2012AA021802) and the Chinese National Natural Science Foundation (#31371265)

REFERENCES

Anand-Srivastava MB (2005). Natriuretic peptide receptor-C signaling and regulation. *Peptides* 26: 1044-1059.

Barrett JC, Fry B, Maller J and Daly MJ (2005). Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265.

Dupont WD and Plummer WD Jr. (1998). Power and sample size calculations for studies involving linear regression. *Control. Clin. Trials* 19: 589-601.

Hunt SC, Hasstedt SJ, Kuida H, Stults BM, et al. (1989). Genetic heritability and common environmental components of resting and stressed blood pressures, lipids, and body mass index in Utah pedigrees and twins. *Am. J. Epidemiol.* 129: 625-638.

International Consortium for Blood Pressure Genome-Wide Association Studies, Ehret GB, Munroe PB, Rice KM, et al. (2011). Genetic variants in novel pathways influence blood pressure and cardiovascular disease risk. *Nature* 478: 103-109.

International HapMap Consortium, Frazer KA, Ballinger DG, Cox DR, et al. (2007). A second generation human haplotype map of over 3.1 million SNPs. *Nature* 449: 851-861.

Kato N, Takeuchi F, Tabara Y, Kelly TN, et al. (2011). Meta-analysis of genome-wide association studies identifies

common variants associated with blood pressure variation in east Asians. *Nat. Genet.* 43: 531-538.
Kearney PM, Whelton M, Reynolds K, Muntner P, et al. (2005). Global burden of hypertension: analysis of worldwide data. *Lancet* 365: 217-223.
Kunes J and Zicha J (2009). The interaction of genetic and environmental factors in the etiology of hypertension. *Physiol. Res.* 58: S33-41.
Pagano M and Anand-Srivastava MB (2001). Cytoplasmic domain of natriuretic peptide receptor C constitutes Gi activator sequences that inhibit adenylyl cyclase activity. *J. Biol. Chem.* 276: 22064-22070.
Purcell S, Neale B, Todd-Brown K, Thomas L, et al. (2007). PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am. J. Hum. Genet.* 81: 559-575.
Sun H, Zhou C, Huang X, Lin K, et al. (2013). Autosomal STRs provide genetic evidence for the hypothesis that Tai people originate from Southern China. *PLoS One* 8: e60822.
Widmaier EP, Raff H, Strang KT and Vander AJ (2008). Vander's Human physiology. McGraw-Hill Higher Education, New York.
World Health Organization (1999). 1999 World Health Organization-International Society of Hypertension Guidelines for the Management of Hypertension. Guidelines Subcommittee. *J. Hypertens.* 17: 151-183.
Xu RW and Yan WL (2013). Advances in genome-wide association studies on essential hypertension. *Yi Chuan* 34: 793-809.