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**ORIGINAL**

# Blood pressure and urinary sodium excretion in relation to 16 genetic polymorphisms in the natriuretic peptide system in Chinese

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**Abstract.** We systematically investigated the association between single nucleotide polymorphisms (SNPs) in the natriuretic peptide system (NPPA, NPPB, NPPC, NPRA, NPRC, and Corin genes) and blood pressure in a Chinese population. The study population was recruited from a mountainous area 500 km south of Shanghai from 2003 to 2009. Using the ABI SNapShot method, we first genotyped 951 subjects enrolled in 2005 for 16 SNPs and then the remaining 1355 subjects as validation for 5 SNPs selected from the primary study. Overall, the association of the studied genetic polymorphisms with blood pressure and urinary excretion of cations was weak or non-significant. However, in the primary study, there was significant ($P_{int}$=0.003) interaction between the rs198358 polymorphism and age in relation to diastolic blood pressure. After adjustment for covariates, diastolic blood pressure was significantly higher in the G allele carriers than AA homozygotes in 176 subjects aged 60 years or older (77.8±1.72 *vs* 73.9±1.54 mmHg, *P*=0.001). In the primary combined with validation studies, this interaction remained statistically significant ($P_{int}$=0.02). The odds ratio of hypertension for carrying the G allele *versus* AA homozygotes was 1.25 (95% CI: 1.03-1.52; *P*=0.03) in all subjects, and 0.85 (0.51-1.41; *P*=0.53), 1.30 (0.98-1.73; *P*=0.06), and 1.45 (0.95-2.22; *P*=0.08) in the subjects younger than 40 years, 40-59 years, and 60 years or older, respectively. Some of the genetic polymorphisms in the natriuretic peptide system might be associated with blood pressure. However, not only the size, but also the direction of the association may change with age.

*Key words:* Genetics, Natriuretic peptides, Blood pressure, Urinary sodium excretion, Population

**NATRIURETIC PEPTIDES** are a family of hormones that include three known peptides: atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and C-type natriuretic peptide (CNP). These three natriuretic peptides have similar chemical structure, and share biological functions [1]. Natriuretic peptides mediated their biological effects mainly by binding to two guanylyl cyclase-coupled receptors (natriuretic peptide receptors A [NPRA] and B [NPRB]) [2]. ANP and BNP preferentially bind to NPRA, and CNP has a higher affinity with NPRB. Natriuretic peptide receptor C (NPRC) behaves as a clearance receptor, which is involved in the degradation of circulating natriuretic peptides. Corin is a recently identified transmembrane serine protease, is highly expressed in cardiomyocytes, and cleaves inactive proANP and proBNP into smaller biologically active molecules [3, 4].

It has been well documented that natriuretic peptides play important roles in the regulation of blood pressure through their diuretic, natriuretic, and vasodilatory properties [5]. In the transgenic mice, overexpression of ANP leads to decreased blood pressure levels [6, 7], whereas in knockout mouse models, lack of ANP [8], NPRA [9], or Corin gene [10] contributes to the development of hypertension. In humans, the infusion of high doses of ANP significantly reduced mean blood pressure, with a marked increase in urinary sodium excretion [11]. In White populations, recent investigations revealed associations of several polymorphisms at the NPPA-NPPB locus with circulating natriuretic peptides [12-14], incident hypertension [12, 13,

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15, 16], and response to diuretic therapy [17]. In addition, the minor alleles of two highly linked polymorphisms (T555I/Q568P) in the Corin gene were reported to be associated with increased risk of hypertension and enhanced cardiac hypertrophic response to pressure overload in African Americans [18, 19]. However, to date, few studies have investigated the relationship between gene variants of the natriuretic peptide system and cardiovascular phenotypes in Chinese [20, 21], in which the dietary sodium intake is high and potassium intake is low, with a urinary $Na^+/K^+$ ratio as high as 6 to 10 [22, 23]. In the present study, we therefore systematically investigated the association of 16 SNPs of 6 genes (NPPA, NPPB, NPPC, NPRA, NPRC, and Corin) with blood pressure and urinary sodium excretion, and explored the potential gene-environment interaction in relation to blood pressure in a Chinese population.

## Methods

### *Study populations*

The study population was recruited in the framework of an ongoing longitudinal population-based genetic study in hypertension [23-25]. From 2003 to 2009, we visited all homes in 18 villages randomly selected from JingNing County, a mountainous rural area approximately 500 km south of Shanghai. We invited all inhabitants at least 12 years of age to take part. Of the 3486 invited, 2666 (76.5%) participated. The study protocol was approved by the Ethics Committee of Ruijin Hospital, Shanghai Jiaotong University School of Medicine, Shanghai, China. All participants gave informed written consent.

Our primary genetic study included 981 subjects enrolled in 2005, for whom we genotyped 16 SNPs in the natriuretic peptide system. We excluded 30 subjects from the present analysis because of missing information on genotype (n=8) and phenotype (n=22). Thus, the total number of subjects in the primary study was 951. Our validation genetic study included the remaining 1685 subjects, for whom we genotyped 5 SNPs selected from the primary study. We excluded 330 subjects because of missing information on genotype (n=12) and phenotype (n=318). Thus, the total number of subjects included in the validation study was 1355.

### *Field work*

One experienced physician measured each participant's brachial blood pressure five times consecutively by mercury sphygmomanometry, after the subject had rested for at least 5 minutes in the sitting position. These five blood pressure readings were averaged for analysis. During the home visit, the same observer administered a standardized questionnaire to collect information on medical history, smoking and drinking habits, and the use of medications. Hypertension was defined as a blood pressure of at least 140 mmHg systolic or 90 mmHg diastolic, or as the use of antihypertensive drugs. A trained technician measured body weight and body height. Body surface area was calculated from body weight and body height using a published formula [26].

Venous blood samples were taken after overnight fasting for the measurement of plasma glucose concentration and for measurements of serum concentrations of creatinine, total cholesterol and triglycerides. Estimated glomerular filtration rate (eGFR) was calculated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [27, 28]. Renal dysfunction was defined as an eGFR below 60 mL/min×1.73 $m^2$. A 24-hour urine sample was collected in a wide-neck plastic container for the measurement of urinary electrolytes. To ensure the complete collection of urine, urinary samples less than 600 mL were excluded. Thus, 847 and 918 urinary samples were available for the measurement of urinary electrolytes in the primary and validation studies, respectively.

### *Genotyping*

The 16 SNPs in 6 genes (NPPA, NPPB, NPPC, NPRA, NPRC, and Corin) of the natriuretic peptide system were genotyped using the ABI PRISM® SNapShot® method (Applied Biosystems, California, USA). In brief, the SNapShot reaction was carried out in a 10 μL final volume containing SNapShot Multiplex Ready Mix (5μL), primer mix (0.02-0.6 μmol/L), and templates (4μL) consisting of the multiplex PCR products, which had been purified with the QIAquick PCR Purification Kit (QIAGEN, GmbH, Germany). The cycling program included 25 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 30 seconds. Extension products were purified by a 15-min incubation with 1 U of shrimp alkaline phosphatase (Promega, Madison, Wisconsin, USA) at 37 °C and a subsequent 15-min incubation at 80 °C to inactivate the enzyme. The purified products (0.5 μL) were mixed with 9 μL of formamide and 0.5 μL of GeneScan-120 LIZ Size Standard (Applied Biosystems) and separated

by capillary electrophoresis (ABI PRISM310 Genetic Analyzer; Applied Biosystems). The results were analyzed with GeneMapper 3.0 software (Applied Biosystems). All PCR and SNapShot primers are listed in supplementary Table 1. More than 98% of the total samples were successfully genotyped for all SNPs. To confirm the genotyping results, 30 samples were randomly selected and re-genotyped by direct sequencing using a BigDye terminator (Applied Biosystems). All assays were 100% concordant.

### *Measurement of plasma proBNP concentration*

In 1386 individuals of the JingNing study from 2003 to 2005, we collected venous blood samples in chilled ethylenediaminetetraacetic acid tubes. After centrifugation, the clarified plasma samples were frozen, stored at -80 °C, and thawed just before assays. Plasma proBNP was measured using an automated, commercially available immunoassay (Elecsys proBNP, Roche Diagnostics, Indianapolis, Indiana, USA) according to manufacturer's instruction. The interassay coefficient of variation in our study was 1.2%.

### *Statistical methods*

We used SAS version 9.13 (SAS institute, Cary, North Carolina, USA) for database management and statistical analyses. Comparisons of means and proportions relied on the standard normal z-test and Fisher's exact test, respectively. Continuous measurements with a skewed distribution were normalized by logarithmic transformation and represented by geometric mean and 95% confidence interval (CI). Construction of linkage disequilibrium map and haplotype blocks with the SNPs at the NPPA-NPPB locus was based on Haploview software version 4.0 (Broad Institute, Cambridge, Massachusetts, USA). Haplotype-based association with hypertension was performed using the Haplo.stats program. We identified covariates of the phenotypes under study using stepwise multiple regressions with *P*-value for independent variables to enter and stay in the model set at 0.10. We studied genetic associations using the analysis of covariance, while controlling for covariates. We did not perform adjustment for multiple comparisons because our analyses were based on related clinical and biochemical measurements or observations. The value of $P<0.05$ was considered to indicate statistical significance.

## Results

### *Characteristics of the participants*

In the primary study, the 951 participants included 470 men (49.4%) and 222 (23.3%) hypertensive patients, of whom 60 (27.0%) took antihypertensive medication. Table 1 shows the characteristics of the study participants by sex. Men and women had similar ($P\geq0.13$) systolic (±standard deviation; 129.5±25.5 mmHg) and diastolic blood pressure (75.8±13.2 mmHg), pulse pressure (53.6±20.1mmHg), serum total cholesterol (4.82±0.97 mmol/L), fasting plasma glucose (4.42±1.37 mmol/L), and urinary sodium (179.2±82.9 mmol/day) and potassium excretions (24.8±9.9 mmol/day). However, men, compared with women, were older (+3.0 years, $P=0.003$), and had higher serum concentrations of triglycerides (+0.24 mmol/L, $P=0.02$) and creatinine (+14.2 mmol/L, $P<0.001$), and urinary volume (+0.19 L, $P<0.001$).

**Table 1** Characteristics of the participants

| Characteristic | Men (n=470) | Women (n=481) | *P* |
|---|---|---|---|
| Age (years) | 46.5±15.9 | 43.5±15.0 | 0.003 |
| Body mass index (kg/m$^2$) | 22.0±2.82 | 22.1±2.93 | 0.46 |
| Body surface area (m$^2$) | 1.61±0.15 | 1.44±0.14 | <0.001 |
| Current smoking, n (%) | 285 (60.6) | 0 | <0.0001 |
| Alcohol intake ≥ 5g/week, n (%) | 297 (63.2) | 139 (28.9) | <0.0001 |
| Prevalence of hypertension (%) | 98 (20.9) | 124 (25.8) | 0.08 |
| Taking antihypertensive drugs (%) | 20 (4.2) | 40 (8.3) | <0.0001 |
| Serum total cholesterol (mmol/L) | 4.87±0.96 | 4.78±0.97 | 0.13 |
| Serum triglycerides (mmol/L) | 1.48±2.08 | 1.24±0.95 | 0.02 |
| Fasting plasma glucose (mmol/L) | 4.38±1.76 | 4.45±0.82 | 0.40 |
| Serum creatinine (mmol/L) | 78.7±22.0 | 64.5±15.1 | <0.001 |
| Estimated GFR (mL/min/1.73 m²) | 86.5±24.2 | 98.7±22.9 | <0.001 |
| Blood pressure (mm Hg) | | | |
| Systolic | 129.2±24.4 | 129.8±26.6 | 0.74 |
| Diastolic | 75.9±12.4 | 75.8±13.9 | 0.88 |
| Pulse pressure | 53.3±20.5 | 54.0±19.7 | 0.60 |
| 24-hour urinary measurements | | | |
| Volume (l) | 1.33±0.58 | 1.14±0.45 | <0.0001 |
| Sodium excretion (mmol/day) | 181.0±86.6 | 178.0±78.7 | 0.54 |
| Potassium excretion (mmol/day) | 25.4±9.7 | 24.4±10.0 | 0.14 |

Values are mean±SD, or number of subjects (percentage of the column total). *P* values for gender difference were calculated by the standard normal z-test (means) or Fisher's exact test (proportions). Body surface area was calculated from body weight and body height using a published formula [26]. Estimated glomerular filtration rate (GFR) was calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) formula [27, 28].

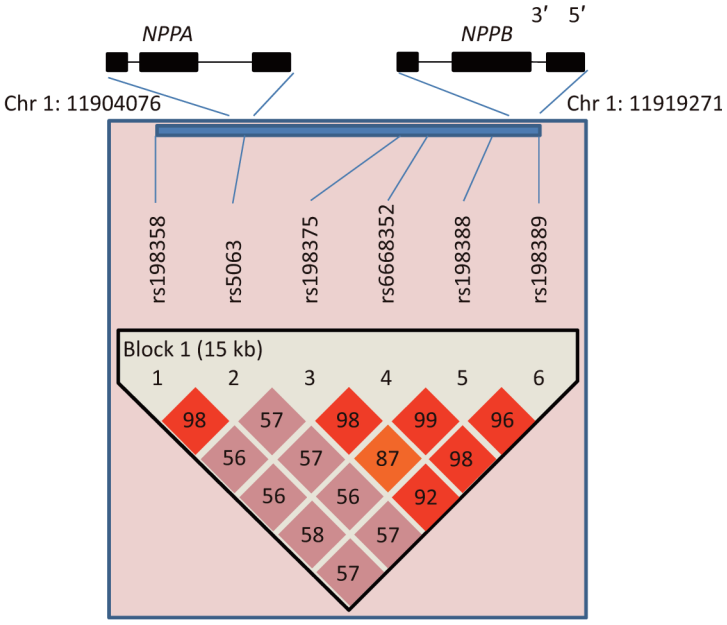


**Fig. 1** Linkage disequilibrium mapping across 6 SNPs (rs198358, rs5063, rs198375, rs6668352, rs198388, and rs198389) at the NPPA-NPPB locus in the primary study. The exon structures of NPPA and NPPB are shown schematically relative to the genotyped SNPs. The horizontal blue frame represents the 15.2 kb region of chromosome 1 analyzed in our study. A linkage disequilibrium plot is depicted based on the measure D' at the bottom of the figure, each diamond represents the pairwise magnitude of linkage disequilibrium, with red and rufous colours indicating strong and weak linkage disequilibrium, respectively. Figure was drawn using Haploview v 4.1.

***Genotype frequencies and linkage disequilibrium***

The genotype frequencies of the 16 SNPs did not deviate from the Hardy-Weinberg equilibrium ($P \geq 0.08$), and were similar in men and women ($P \geq 0.12$). In single SNP analyses, no significant ($P \geq 0.06$) differences in genotype distribution were found between hypertensive patients and normotensive subjects (supplementary Table 2). In multiple SNP analyses, of the 6 SNPs at the NPPA-NPPB locus, we revealed two highly structured linkage disequilibrium blocks (l*D*'l>0.8), a major linkage disequilibrium block (rs198389, rs198388, rs6668352, and rs198375) and a minor linkage disequilibrium block (rs5063 and rs198358) as shown in Fig. 1. In haplotype analyses, five haplotypes had a frequency >1% and accounted for >95% of all haplotypes. However, none of the associations between the 5 haplotypes and the prevalence of hypertension reached statistical significance ($P \geq 0.39$, supplementary Table 3).

***Blood pressure and SNPs in the natriuretic peptide system***

In the primary study, after adjustment for age, sex, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs, of the 16 SNPs, the minor alleles of the rs198375 and rs6668352 polymorphisms at the NPPA-NPPB locus were significantly associated with higher diastolic blood pressure ($P \leq 0.05$), while the minor alleles of the rs700923 and rs2292026 polymorphisms, which were in tight linkage disequilibrium (r'=0.88) in the NPRC gene, were associated with lower pulse pressure ($P \leq 0.02$, Table 2 and supplementary Table 4). With adjustments for age, sex, body mass index, body surface area, serum creatinine, and the use of antihypertensive drugs, we also found that the minor alleles of the rs3811544 (NPPC) and rs198358 polymorphisms (NPPA) were significantly associated with lower urinary sodium excretion ($P \leq 0.03$, Table 2).

Further analyses revealed that there was significant ($P_{int}=0.003$) interaction between the rs198358 polymorphism and age in relation to diastolic blood pressure. The genetic effect of the rs198358 polymorphism on diastolic blood pressure became more prominent with advancing age (Fig. 2). After 40 years of age, diastolic blood pressure remained at plateau level in the G allele carriers, but had a significant decrease with age in AA homozygotes. Accordingly, in 176 subjects of 60

**Table 2** Blood pressure and urinary electrolytes excretions in relation to five polymorphisms of the natriuretic peptide system in the primary study

| Gene and polymorphism | Systolic pressure (mmHg) | | Diastolic pressure (mmHg) | | Pulse pressure (mmHg) | | Urinary volume (l) | | Urinary sodium excretion (mmol/day) | | Urinary potassium excretion (mmol/day) | | $Na^+/K^+$ ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ |
| rs198358 (NPPA-NPPB) | | | | | | | | | | | | | | |
| AA ( n=697) | 129.5±0.81 | | 75.7±0.50 | | 53.9±0.64 | | 1.26±0.02 | | 183.3±3.22 | | 25.4±0.38 | | 7.47±0.11 | |
| GA ( n=237) | 129.4±1.35 | | 76.4±0.83 | | 53.0±1.07 | | 1.26±0.04 | | 174.4±5.37 | | 24.1±0.64 | | 7.58±0.19 | |
| GG ( n=17) | 136.2±5.17 | 0.44 | 80.0±3.19 | 0.32 | 56.2±4.09 | 0.64 | 1.01±0.15 | 0.24 | 130.6±23.2 | 0.03 | 21.4±2.76 | 0.04 | 7.29±0.81 | 0.58 |
| rs6668352 (NPPA-NPPB) | | | | | | | | | | | | | | |
| GG ( n=674) | 129.7±0.82 | | 75.3±0.50 | | 54.4±0.64 | | 1.25±0.02 | | 182.2±3.28 | | 25.2±0.39 | | 7.49±0.11 | |
| GA ( n=262) | 129.5±1.30 | | 77.5±0.80 | | 52.0±1.02 | | 1.24±0.03 | | 173.4±5.15 | | 24.3±0.61 | | 7.47±0.18 | |
| AA ( n=15) | 128.3±5.55 | 0.96 | 78.4±3.41 | 0.04 | 49.9±4.38 | 0.10 | 1.81±0.15 | 0.007 | 220.3±23.1 | 0.10 | 29.5±2.75 | 0.13 | 7.87±0.81 | 0.89 |
| rs3811544 (NPPC) | | | | | | | | | | | | | | |
| CC (n=902) | 129.8±0.71 | | 76.1±0.44 | | 53.7±0.56 | | 1.24±0.02 | | 178.4±2.83 | | 24.8±0.34 | | 7.48±0.10 | |
| CT+TT (n=49) | 129.0±2.98 | 0.80 | 73.6±1.85 | 0.19 | 55.2±2.36 | 0.55 | 1.39±0.08 | 0.04 | 205.8±11.6 | 0.02 | 26.6±1.38 | 0.21 | 7.69±0.41 | 0.62 |
| rs700923 (NPRC) | | | | | | | | | | | | | | |
| AA (n=586) | 130.8±0.88 | | 75.7±0.54 | | 55.1±0.69 | | 1.24±0.02 | | 179.7±3.56 | | 24.7±0.42 | | 7.51±0.12 | |
| GA (n=332) | 128.8±1.17 | | 76.5±0.72 | | 52.2±0.92 | | 1.25±0.03 | | 179.4±4.65 | | 25.2±0.55 | | 7.51±0.16 | |
| GG (n=33) | 125.2±3.61 | 0.17 | 75.6±2.23 | 0.60 | 49.6±2.85 | 0.01 | 1.37±0.10 | 0.43 | 194.8±14.7 | 0.59 | 27.5±1.74 | 0.26 | 7.46±0.51 | 0.99 |
| rs9662664 (NPRA) | | | | | | | | | | | | | | |
| GG(n=442) | 129.9±1.03 | | 75.9±0.64 | | 53.9±0.82 | | 1.23±0.03 | | 184.5±4.12 | | 25.4±0.49 | | 7.56±0.14 | |
| GT (n=420) | 129.2±1.04 | | 76.0±0.65 | | 53.2±0.83 | | 1.26±0.03 | | 172.8±4.16 | | 24.2±0.49 | | 7.41±0.15 | |
| TT (n=109) | 131.7±2.04 | 0.56 | 76.4±1.26 | 0.93 | 55.4±1.61 | 0.46 | 1.28±0.05 | 0.68 | 192.0±8.35 | 0.06 | 26.3±0.99 | 0.09 | 7.65±0.29 | 0.67 |

Values are mean±SE. Systolic and diastolic blood pressure and pulse pressure were adjusted for sex, age, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs. Urinary electrolytes were measured in 847 individuals. Urinary sodium and potassium excretions were adjusted for sex, age, body mass index, serum creatinine, body surface area, and the use of antihypertensive drugs. *On the basis of analysis of variance.

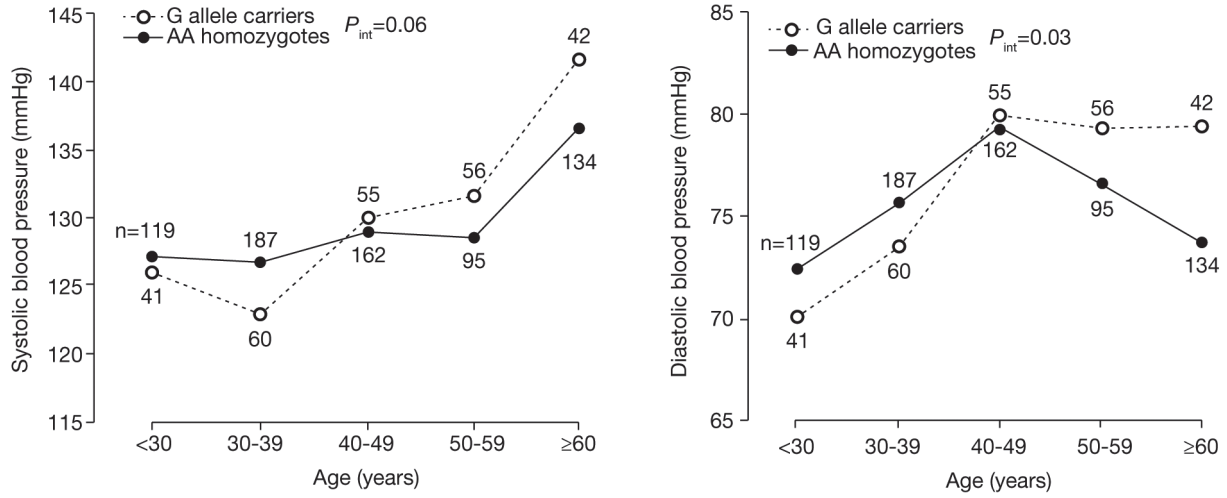


**Fig. 2** Systolic (left) and diastolic (right) blood pressure by age and the rs198358 genotype. Values are mean, adjusted for sex, age, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs. The *P*-value for interaction ($P_{int}$) between the rs198358 genotype and age as a continuous variable was derived from the analysis of covariance. For each genotype, the number of subjects is given.

**Table 3** Blood pressure and urinary electrolytes excretions in relation to five polymorphisms of the natriuretic peptide system in the validation study

| Gene | Systolic pressure (mmHg) | | Diastolic pressure (mmHg) | | Pulse pressure (mmHg) | | Urinary volume (l) | | Urinary sodium excretion (mmol/day) | | Urinary potassium excretion (mmol/day) | | $Na^+/K^+$ ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean±SE | $P^*$ | Mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ |
| rs198358 (NPPA-NPPB) | | | | | | | | | | | | | | |
| AA (n=927) | 123.4±0.62 | | 76.7±0.35 | | 46.7±0.45 | | 1.11±0.02 | | 140.9±2.41 | | 23.6±0.41 | | 6.42±0.09 | |
| GA (n=403) | 126.2±0.95 | | 78.0±0.54 | | 48.4±0.69 | | 1.14±0.03 | | 140.8±3.7 | | 23.1±0.63 | | 6.47±0.14 | |
| GG (n=25) | 126.3±3.86 | 0.03 | 80.5±2.20 | 0.04 | 45.8±2.83 | 0.11 | 1.03±0.10 | 0.40 | 118.3±14.6 | 0.31 | 19.7±2.51 | 0.28 | 6.47±0.56 | 0.86 |
| rs6668352 (NPPA-NPPB) | | | | | | | | | | | | | | |
| GG (n=864) | 123.9±0.65 | | 77.0±0.37 | | 46.8±0.47 | | 1.11±0.02 | | 139.2±2.51 | | 23.2±0.43 | | 6.45±0.10 | |
| GA (n=446) | 125.3±0.91 | | 77.3±0.52 | | 48.0±0.66 | | 1.11±0.03 | | 141.4±3.44 | | 23.5±0.59 | | 6.44±0.13 | |
| AA (n=45) | 124.3±2.91 | 0.46 | 78.2±1.66 | 0.74 | 46.0±2.13 | 0.33 | 1.10±0.08 | 0.99 | 149.5±11.3 | 0.62 | 24.8±1.95 | 0.62 | 6.28±0.43 | 0.93 |
| rs3811544 (NPPC) | | | | | | | | | | | | | | |
| CC (n=1273) | 124.5±0.53 | | 77.3±0.30 | | 47.3±0.39 | | 1.11±0.01 | | 140.2±2.05 | | 23.4±0.35 | | 6.43±0.08 | |
| CT+TT (n=82) | 121.0±2.11 | 0.11 | 74.9±1.21 | 0.06 | 44.8±1.53 | 0.12 | 1.16±0.06 | 0.44 | 145.1±8.71 | 0.58 | 23.0±1.50 | 0.81 | 6.70±0.33 | 0.42 |
| rs700923 (NPRC) | | | | | | | | | | | | | | |
| AA (n=849) | 125.0±0.64 | | 77.5±0.37 | | 47.5±0.47 | | 1.12±0.02 | | 141.9±2.54 | | 23.6±0.44 | | 6.46±0.10 | |
| GA (n=457) | 123.0±0.88 | | 76.3±0.51 | | 46.7±0.65 | | 1.10±0.02 | | 137.7±3.40 | | 23.1±0.58 | | 6.36±0.13 | |
| GG (n=49) | 121.1±2.71 | 0.10 | 77.8±1.56 | 0.11 | 43.2±1.98 | 0.10 | 1.18±0.08 | 0.60 | 139.1±10.5 | 0.59 | 22.5±1.80 | 0.73 | 6.80±0.40 | 0.54 |
| rs9662664 (NPRA) | | | | | | | | | | | | | | |
| GG(n=587) | 123.1±0.78 | | 76.6±0.45 | | 46.5±0.57 | | 1.11±0.02 | | 141.7±2.98 | | 23.2±0.51 | | 6.48±0.11 | |
| GT (n=601) | 125.4±0.77 | | 77.4±0.44 | | 48.0±0.56 | | 1.10±0.02 | | 140.6±3.06 | | 23.6±0.53 | | 6.45±0.12 | |
| TT (n=167) | 124.0±1.47 | 0.12 | 78.0±0.85 | 0.23 | 45.9±1.08 | 0.11 | 1.16±0.04 | 0.48 | 135.1±5.77 | 0.60 | 23.1±0.99 | 0.87 | 6.19±0.22 | 0.48 |

Values are mean±SE. Systolic and diastolic blood pressure and pulse pressure were adjusted for sex, age, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs. Urinary electrolytes were measured in 918 individuals. Urinary sodium and potassium excretions were adjusted for sex, age, body mass index, serum creatinine, body surface area, and the use of antihypertensive drugs. *On the basis of analysis of variance.

years or older, diastolic blood pressure was significantly higher in the G allele carriers than AA homozygotes (77.8±1.72 mmHg *vs* 73.9±1.54 mmHg, $P$=0.001). A similar trend was observed for the age-genotype interaction on systolic blood pressure ($P_{int}$=0.06).

***The validation study***

We performed a validation study in the remaining 1355 individuals of the JingNing study. Of the 5 SNPs genotyped (rs198358, rs6668352, rs3811544, rs700923, and rs9662664), only the rs198358 polymorphism was significantly associated with systolic and diastolic blood pressure ($P$≤0.04, Table 3) and the prevalence of hypertension, but not with urinary electrolyte excretion ($P$≥0.23, Table 3). The G allele carriers, compared with AA homozygotes, had higher systolic (126.4±0.92 mmHg *vs* 123.4±0.62 mmHg; $P$=0.007) and diastolic blood pressure (78.1±0.52 mmHg *vs* 76.7±0.35 mmHg; $P$=0.02) and an increased risk of hypertension (odds ratio 1.33, 95% CI: 1.03-1.72, $P$=0.03).

In the combined analysis of the primary and validation studies, the interaction between the rs198358 polymorphism and age in relation to diastolic blood pressure remained statistically significant ($P_{int}$=0.02), indicating that the association between the rs198358 polymorphism and the risk of hypertension was dependent on age. The odds ratio of hypertension for carrying the G allele *versus* AA homozygotes was 1.25 (95% CI: 1.03-1.52; $P$=0.03) in all subjects (n=2306), and 0.85 (95% CI: 0.51-1.41; $P$=0.53), 1.30 (95% CI: 0.98-1.73; $P$=0.06), and 1.45 (95% CI: 0.95-2.22; $P$=0.08) in the subjects younger than 40 years (n=953), 40-59 years (n=917), and 60 years or older (n=436),

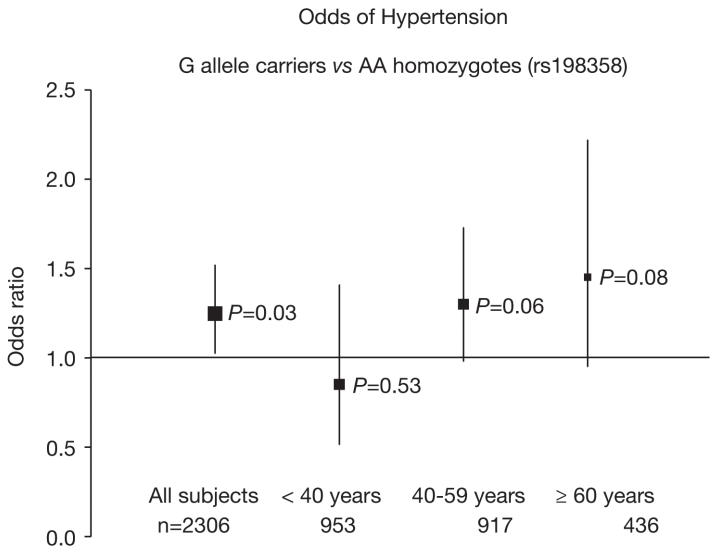


**Fig. 3** Odds ratio of hypertension associated with the rs198358 G allele in the entire study, and in subjects younger than 40 years, 40-59 years, and 60 years or older. Squares are mean, and the size of symbols is proportional to the number of subjects (bottom). Vertical lines denote 95% confidence interval (CI).

respectively (Fig. 3). In addition, the minor alleles of the rs3811544 and rs700923 were significantly associated with lower diastolic blood pressure ($P$=0.008) and pulse pressure ($P$=0.02), respectively (supplementary Table 5). The rs3811544 T allele carriers, compared with CC homozygotes, had significantly lower diastolic blood pressure (74.1±1.01 *vs* 76.8±0.25 mmHg), while the rs700923 G allele carriers, compared with AA homozygotes, had significantly lower pulse pressure (48.7±0.53 mmHg *vs* 50.2±0.41 mmHg).

### *Association between SNPs in the natriuretic peptide system and plasma proBNP level*

We measured plasma proBNP concentration in 1386 subjects enrolled in our JingNing study from 2003 to 2005. We excluded from our analyses 28 subjects with self-reported chronic heart failure (n=22), renal dysfunction (n=5) or both diseases (n=1). Both before and after adjustment for age, sex, body mass index, body surface area, and serum creatinine, the 5 SNPs (rs198358, rs6668352, rs198375, rs198388, and rs198389) at the NPPA-NPPB locus were significantly ($P\leq$0.02) associated with plasma proBNP level. The adjusted geometric mean plasma proBNP concentration was 35.5 pg/mL (95% CI: 33.9 to 37.2), 38.2 pg/mL (95% CI: 35.4 to 41.2), and 49.3 pg/mL (95% CI: 36.2 to 67.2; $P$=0.02) in the AA, GA, and GG subjects of the rs198358 polymorphism, respectively (Table 4).

**Table 4** Plasma NT-proBNP concentration in relation to the polymorphisms at the NPPA-NPPB locus

| Gene and genotype | proBNP (pg/mL) | |
|---|---|---|
| | Geometric mean (95% CI) | $P^*$ |
| rs5063 | | |
| GG (n=667) | 34.5 (32.5 to 36.5) | |
| GA (n=193) | 36.2 (32.6 to 40.2) | |
| AA (n=16) | 32.1 (22.3 to 46.3) | 0.65 |
| rs198358 | | |
| AA (n=979) | 35.5 (33.9 to 37.2) | |
| GA (n=354) | 38.2 (35.4 to 41.2) | |
| GG (n=25) | 49.3 (36.2 to 67.2) | 0.02 |
| rs6668352 | | |
| GG (n=925) | 34.0 (32.4 to 35.6) | |
| GA (n=399) | 41.2 (38.4 to 44.2) | |
| AA (n=34) | 49.8 (39.3 to 63.1) | <0.0001 |
| rs198375 | | |
| AA (n=577) | 32.6 (30.7 to 34.6) | |
| GA (n=273) | 38.6 (35.3 to 42.1) | |
| GG (n=26) | 49.4 (36.2 to 67.2) | 0.001 |
| rs198388 | | |
| CC (n=588) | 32.7 (30.8 to 34.8) | |
| CT (n=265) | 38.9 (36.4 to 42.6) | |
| TT (n=23) | 45.9 (33.9 to 62.2) | 0.001 |
| rs198389 | | |
| AA (n=601) | 32.5 (30.6 to 34.5) | |
| GA (n=250) | 40.0 (36.5 to 43.9) | |
| GG (n=25) | 43.5 (32.5 to 58.2) | 0.0003 |

*Adjusted for age, sex, body mass index and serum creatinine.

## Discussion

Our population-based study demonstrated that at least some of the genetic polymorphisms in the natriuretic peptide system might be associated with blood pressure. However, the size and direction of the association may change with age. Indeed, the rs198358 G allele carriers, compared with AA homozygotes, had a similar or even lower risk of hypertension before 40 years of age, but 30% and 45% higher risk of hypertension in 40-59 years and after 60 years, respectively.

Over the past decade, numerous studies investigated genetic variants in the natriuretic peptide system in relation to hypertension and its related phenotypes [29]. Of these genetic variants, two known missense variants (rs5063 and rs5065) in the NPPA gene have been shown to be associated with hypertension [15, 16], coronary heart disease [30], stroke [31, 32], and response to diuretic therapy [17]. In addition, several other SNPs at the NPPA-NPPB locus (rs198388, rs198389, rs6668352) [33] and the NPPC (rs5268) [34], NPRA (rs28730726) [35] and NPRC genes (rs700923) [33] were reported to be associated with the risk of left ventricular dysfunction after primary coronary artery bypass grafting and with the risk of hypertension.

Our finding that the minor G allele of the rs198358 polymorphism was associated with a higher risk of hypertension seems to be contradictory with the theory that a genetic variant associated with higher levels of natriuretic peptides should lead to a lower blood pressure, and with the results of 2 previous studies that showed a negative association of the G allele with systolic and diastolic blood pressure and the risk of hypertension [12, 13]. In 29717 subjects (mean age from 43 to 58 years) recruited from Finland, Sweden, and USA, the G allele of the rs198358 polymorphism was associated with a lower systolic (-0.05 mmHg) and diastolic blood pressure (-0.05 mmHg) and a lower risk of hypertension (-10%) [12]. In 5212 middle-aged (51 years) French subjects, Maimaitiming *et al.* found that the haplotype GCG harbouring the G allele of the rs198358 polymorphism, with a population frequency of 4.4%, was associated with lower systolic and diastolic blood pressure [13].

The difference between our and previous findings on the rs198358 polymorphism and blood pressure is not entirely understood. The age dependency of the association might be an explanation. In the early years of life, elevated circulating levels of natriuretic peptides due to genetic effect, might have blood pressure lowering actions by increasing urinary excretions of sodium and water. However, if sodium intake is high, such as in the Chinese, a long-term glomerular hyperfiltration in the long run might accelerate decay of renal function, impair sodium and water homeostasis, lead to chronic expansion of circulating fluid volume, and ultimately contribute to the development of hypertension in the elderly. Therefore, we speculate that, in the presence of the rs198358 G allele, the increased diastolic blood pressure in the elderly might be, to some extent, attributable to the deterioration of renal function resulting from prolonged elevation of circulating natriuretic peptides. Indeed, two prospective studies consistently showed that the elevated BNP level predicted an increased risk of accelerated progression from chronic kidney disease to end stage renal dysfunction [36, 37]. Similarly, during on average 9 years of follow up, Maimaitiming *et al.* observed that the subjects carrying the GCG haplotype harbouring the rs198358 G allele had a higher, though non-significant, risk of microalbuminuria (odds ratio 1.27, $P$=0.15) [13]. Furthermore, in our study, the decline in eGFR with age tended to be greater in 42 GG homozygotes than 1624 AA homozygotes (β±SE; -1.32±0.28 *vs* -0.94±0.04 mL/min/1.73 $m^2$ per year, $P$=0.07). In addition, the differences in genotype frequency and lifestyle across ethnicities might also explain differences in genetic associations. The frequencies of the rs198358 G allele significantly differ between Asians (12.5%), Caucasians (30.6%), and African-Americans (49.2%) (http://www.ncbi.nlm.nih.gov/SNP). It is also known that Chinese, compared with other populations, have a higher intake of sodium and a lower intake of potassium, and hence a higher $Na^+/K^+$ ratio. Chinese are also more sensitive in their blood pressure responses to sodium intake, especially in the elderly. A recent study reported that approximately 39% of adult Chinese were sodium sensitive [38]. The influence of the natriuretic peptide system on blood pressure regulation might be affected by an enhanced sodium-sensitivity with age advancing.

Few studies investigated the association between genetic variants of the NPPC and NPRC genes and hypertension. Our study was the first that demonstrated significant associations of the NPPC rs3811544 and NPRC rs700923 polymorphisms with diastolic blood pressure ($P$=0.02) and pulse pressure ($P$=0.02). In a case-control study including 697 patients of European descent undergoing primary coronary artery bypass

graft, the rs700923 minor allele was associated with an increased risk of left ventricular dysfunction [33]. This observation, taken together with our finding, supports that the NPRC rs700923 polymorphism might be a susceptible locus for cardiovascular phenotypes, and warrant further investigation.

Our study should be interpreted within the context of its strengths and limitations. In an extensively phenotyped general population sample, we may systemically test interaction between genetic variants and various environmental factors. In addition, we studied several well-characterized genetic polymorphisms in the natriuretic peptide system. However, our study was cross-sectional, and hence no causal conclusion could be drawn. Second, we did not determine plasma ANP and CNP concentrations, nor explore the functional role of the studied polymorphisms. Third, our study had a relatively small sample size, and the results of the subgroup analysis should be cautiously interpreted.

In conclusion, some of the genetic polymorphisms in the natriuretic peptide system might be associated with blood pressure. However, not only the size, but also the direction of the association may change with age. One of the major implications of our study is that genetic associations with hypertension, an apparent age-related multifactorial chronic disease might be context-dependent even for apparently functional genetic variants.

## Acknowledgments

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## Conflict of Interest

None of the authors have a conflict of interest with regard to the data presented in this paper.

**Supplementary Table 1** PCR and SNaPshot primers of 16 SNPs in the natriuretic peptide system

| Gene | SNP | Forward primer | Reverse primer | SNaPshot primer |
|---|---|---|---|---|
| NPPA-NPPB | | | | |
| | rs198358 | 5'-AACCTGGCTCCTGAGGTCTG-3' | 5'-CCGATGTCCAACCAGAAGAA-3' | 5'-ttttttttttttttttttTGCGACCACTGATGGAAC-3' |
| | rs5063 | 5'-GCTTCTTCATTCGGCTCACT-3' | 5'-GCAGTGGATTGCTCCTTGAC-3' | 5'-tttttttttGCTAATCCCATGTACAATGCC-3' |
| | rs198375 | 5'-GTGGCGCACACCTGTAGTCT-3' | 5'-ATGGCTTCAGAGCTGCAAGA-3' | 5'-ttttttttttttttttttttttttttttttttTTTGCACCTGGGATAGTCTC-3' |
| | rs6668352 | 5'-TTGGGTCCTTTCCCTAAGCA-3' | 5'-CCAGCCTGAGCAACAGAGAA-3' | 5'-ttttttttttttttttttttttttttttttttttttttttttttttttCTCACTGGGTACAGCAGGC-3' |
| | rs198388 | 5'-GAGGATGTGGCCTCAGAGGT-3' | 5'-GAGCAAGGACGAGTGGAGAA-3' | 5'-ttttttttttttttGCCTCAAGTGCTTGAGATATT-3' |
| | rs198389 | 5'-GAGCAGGTGCTGCGCTACG-3' | 5'-CGCGCTGGAGACACAGACA-3' | 5'-ttttttttttttttttttttttTTCCTTTCCTGCAAATGTCC-3' |
| NPPC | | | | |
| | rs5262 | 5'-GCGCGGAACCAAGATATTCA-3' | 5'-GGGAAGTTGACCACCTGTCAC-3' | 5'-ttttttttttttttttttttttttttttttttCGCATTGTTCCCACAGA-3' |
| | rs3811544 | 5'-AGCCGTGACAGGTGGTCAA-3' | 5'-GCTCAGCAGCAGCCAGTCAT-3' | 5'-ttttttttttttttttttttttttttttttttttttttttttttttttAGCCCAGTGCAAGGAGAG-3' |
| NPRA | | | | |
| | rs28730726 | 5'-ACACCTGGCCTATGAGCAGT-3' | 5'-AAGGATGGAGGCTGGCAGA-3' | 5'-ttttttttttttttttttttTGAGCAGTTCAACTTCACCAT-3' |
| | rs9662664 | 5'-GGCCATTCCTATCCTTGTCTC-3' | 5'-CTCTCCTTGCTCGGCATTCT-3' | 5'-ttttttttttttttttttttttttttttttttttGGGACCCGGAGAACCAA-3' |
| NPRC | | | | |
| | rs2270915 | 5'-TGTCCCTTGAGGGCACTCTT-3' | 5'-AAATGGGATCTGATGGAATCTT-3' | 5'-ttttttttttttttttttttttttttttttttttttttttttttttttttttttttGAACCCAGCAAGAAGAAAGT-3' |
| | rs700923 | 5'-GCTGCCTCGCCAGACATGAA-3' | 5'-TCTGCAATGATGGCTATGTTCT-3' | 5'-ttttttttttttGGCATTGAGCACTTTAAATATG-3' |
| | rs2292026 | 5'-CTTCCATGAGCCATCTCCTA-3' | 5'-TTGACGTTGTCCCATCATACCT-3' | 5'-ttttttttttttttttttttttAAAAGTAACATGCGGGGG-3' |
| Corin | | | | |
| | rs11934749 | 5'-TCTGCATGAATTTGCCCTACA-3' | 5'-TCACCCCTTGGTTCTCTCTGA-3' | 5'-ttttttttttttttttttttttttttATTACCTGCAAGGAGGGATA-3' |
| | rs3749585 | 5'-TAAATCTCGCCAGAGTCGATAA-3' | 5'-AAATGGCCTTCTTGACTGTGA-3' | 5'-ttttttttttttttttttttttttttttttttttttttttttttttttGCAAATTTGCATGTTTGTTT-3' |
| | rs2289433 | 5'-GGACTAAGAGGCCATCACACC-3' | 5'-GGACACGGAGGATCTGTCATT-3' | 5'-ttttttttttttttttttttttttttttttttttttttttttttttttGCTCCGGAAGAGCGCT-3' |

**Supplementary Table 2** Genotype frequencies

| Polymorphism | Genotypes | | | $P^*$ | Polymorphism | Genotypes | | | $P^*$ |
|---|---|---|---|---|---|---|---|---|---|
| rs198358 | AA | GA | GG | | rs28730726 | GG | GC | CC | |
| Normotensive subjects, n (%) | 535 (73.4) | 185 (25.4) | 9 (1.2) | | Normotensive subjects, n (%) | 619 (85.1) | 104 (14.3) | 4 (0.6) | |
| Hypertensive patients, n (%) | 162 (73.0) | 52 (23.4) | 8 (3.6) | 0.06 | Hypertensive patients, n (%) | 183 (82.4) | 38 (17.1) | 1 (0.5) | 0.58 |
| rs5063 | GG | GA | AA | | rs9662664 | GG | GT | TT | |
| Normotensive subjects, n (%) | 557 (76.3) | 161 (22.2) | 11 (1.5) | | Normotensive subjects, n (%) | 319 (43.8) | 334 (45.8) | 76 (10.4) | |
| Hypertensive patients, n (%) | 167 (75.1) | 50 (22.6) | 5 (2.3) | 0.74 | Hypertensive patients, n (%) | 103 (46.4) | 86 (38.7) | 33 (14.9) | 0.07 |
| rs198375 | AA | GA | GG | | rs2270915 | AA | GA | GG | |
| Normotensive subjects, n (%) | 475 (65.2) | 236 (32.4) | 18 (2.5) | | Normotensive subjects, n (%) | 532(73.0) | 186 (25.5) | 11 (1.5) | |
| Hypertensive patients, n (%) | 147 (66.2) | 68 (30.6) | 7 (3.2) | 0.78 | Hypertensive patients, n (%) | 161 (72.5) | 56 (25.2) | 5 (2.3) | 0.75 |
| rs6668352 | GG | GA | AA | | rs700923 | AA | GA | GG | |
| Normotensive subjects, n (%) | 514 (70.5) | 204 (28.0) | 11 (1.5) | | Normotensive subjects, n (%) | 448 (61.5) | 257 (35.3) | 24 (3.2) | |
| Hypertensive patients, n (%) | 160 (72.1) | 58 (26.1) | 4 (1.8) | 0.83 | Hypertensive patients, n (%) | 138 (62.2) | 75 (33.8) | 9 (4.1) | 0.81 |
| rs198388 | CC | CT | TT | | rs2292026 | CC | CT | TT | |
| Normotensive subjects, n (%) | 483 (66.3) | 228 (31.2) | 18 (2.5) | | Normotensive subjects, n (%) | 463 (63.5) | 244 (33.5) | 22 (3.0) | |
| Hypertensive patients, n (%) | 153 (68.9) | 63 (28.4) | 6 (2.7) | 0.71 | Hypertensive patients, n (%) | 142 (64.0) | 72 (32.4) | 8 (3.6) | 0.88 |
| rs198389 | AA | GA | GG | | rs11934749 | CC | CT | | |
| Normotensive subjects, n (%) | 491 (67.4) | 219 (30.0) | 19 (2.6) | | Normotensive subjects, n (%) | 712 (97.7) | 17 (2.3) | 0 | |
| Hypertensive patients, n (%) | 156 (70.3) | 60 (27.0) | 6 (2.7) | 0.69 | Hypertensive patients, n (%) | 217 (97.7) | 5 (2.3) | 0 | 0.94 |
| rs5262 | GG | GA | AA | | rs3749585 | TT | CT | CC | |
| Normotensive subjects, n (%) | 521 (71.4) | 185 (25.4) | 23 (3.2) | | Normotensive subjects, n (%) | 247 (33.9) | 337 (46.2) | 145 (19.9) | |
| Hypertensive patients, n (%) | 153 (68.9) | 66 (29.7) | 3 (1.4) | 0.18 | Hypertensive patients, n (%) | 82 (36.9) | 99 (44.6) | 41 (18.5) | 0.69 |
| rs3811544 | CC | CT | TT | | rs2289433 | AA | GA | GG | |
| Normotensive subjects, n (%) | 695 (95.3) | 34 (4.7) | 0 | | Normotensive subjects, n (%) | 361 (49.5) | 298 (40.9) | 70 (9.6) | |
| Hypertensive patients, n (%) | 207 (93.2) | 15 (6.8) | 0 | 0.22 | Hypertensive patients, n (%) | 109 (49.1) | 99 (44.6) | 14 (6.3) | 0.26 |

Values in the parentheses are percentage of the column total.
*Fisher's exact test was used to compare hypertensive patients with normotensive subjects.

**Supplementary Table 3** Association between haplotypes constructed with 6 SNPs at the NPPA-NPPB locus and the risk of hypertension

| n | Haplotype | Frequency | Haplotype frequency | | Odds ratio (95% confidence interval) | *P* |
|---|---|---|---|---|---|---|
| | | | Hypertensive | Normotensive | | |
| 1 | A-G-A-G-C-A | 0.745 | 0.744 | 0.746 | 1 | reference |
| 2 | G-A-G-A-T-G | 0.081 | 0.088 | 0.079 | 1.09 (0.73-1.62) | 0.68 |
| 3 | A-G-G-A-T-G | 0.060 | 0.049 | 0.064 | 0.77 (0.46-1.28) | 0.31 |
| 4 | G-A-A-G-C-A | 0.041 | 0.047 | 0.039 | 1.20 (0.69-2.09) | 0.51 |
| 5 | A-G-G-G-C-A | 0.020 | 0.026 | 0.017 | 1.43 (0.70-2.92) | 0.32 |

**Supplementary Table 4** Blood pressure and urinary electrolytes excretions in relation to eleven polymorphisms of the natriuretic peptide system in the primary study

| Gene and polymorphism | Systolic pressure (mmHg) | | Diastolic pressure (mmHg) | | Pulse pressure (mmHg) | | Urinary volume (l) | | Urinary sodium excretion (mmol/day) | | Urinary potassium excretion (mmol/day) | | $Na^+/K^+$ ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ |
| rs5063 (NPPA-NPPB) | | | | | | | | | | | | | | |
| GG (n=724) | 129.7±0.79 | | 75.9±0.49 | | 55.5±4.24 | | 1.26±0.02 | | 183.9±3.17 | | 25.2±0.38 | | 7.53±0.11 | |
| GA (n=211) | 129.5±1.43 | | 76.2±0.88 | | 53.3±1.14 | | 1.24±0.04 | | 171.0±5.68 | | 24.3±0.68 | | 7.45±0.20 | |
| AA (n=16) | 133.5±5.35 | 0.77 | 78.0±3.30 | 0.78 | 53.8±0.62 | 0.85 | 1.20±0.14 | 0.82 | 139.9±22.1 | 0.06 | 23.6±2.64 | 0.40 | 6.77±0.78 | 0.53 |
| rs198375 (NPPA-NPPB) | | | | | | | | | | | | | | |
| AA (n=622) | 129.4±0.85 | | 75.4±0.52 | | 53.9±0.67 | | 1.24±0.02 | | 181.0±3.42 | | 25.2±0.41 | | 7.43±0.12 | |
| GA (n=304) | 129.9±1.20 | | 76.6±1.20 | | 53.3±0.96 | | 1.26±0.03 | | 178.2±4.82 | | 24.6±0.57 | | 7.58±0.17 | |
| GG (n=25) | 132.0±4.17 | 0.80 | 80.8±2.56 | 0.05 | 51.2±3.30 | 0.65 | 1.34±0.11 | 0.65 | 187.1±16.4 | 0.82 | 25.6±1.94 | 0.68 | 7.62±0.57 | 0.75 |
| rs198388 (NPPA-NPPB) | | | | | | | | | | | | | | |
| CC (n=636) | 129.5±0.84 | | 75.4±0.52 | | 54.1±0.67 | | 1.25±0.02 | | 183.4±3.38 | | 25.3±0.40 | | 7.51±0.12 | |
| CT (n=291) | 130.4±1.23 | | 77.2±0.76 | | 53.2±0.98 | | 1.23±0.03 | | 172.0±4.92 | | 24.3±0.59 | | 7.44±0.17 | |
| TT (n=24) | 125.3±4.26 | 0.49 | 75.8±2.62 | 0.15 | 49.5±3.37 | 0.33 | 1.61±0.11 | 0.004 | 195.9±17.6 | 0.11 | 26.2±2.09 | 0.37 | 7.88±0.62 | 0.78 |
| rs198389 (NPPA-NPPB) | | | | | | | | | | | | | | |
| AA (n=647) | 129.6±0.84 | | 75.3±0.51 | | 54.3±0.66 | | 1.26±0.02 | | 182.3±3.35 | | 25.2±0.40 | | 7.48±0.12 | |
| GA (n=279) | 130.2±1.26 | | 77.3±0.78 | | 52.9±1.01 | | 1.23±0.03 | | 175.1±5.07 | | 24.4±0.60 | | 7.54±0.18 | |
| GG (n=25) | 125.6±4.27 | 0.57 | 77.1±2.62 | 0.09 | 48.5±3.38 | 0.16 | 1.46±0.11 | 0.15 | 184.0±17.7 | 0.48 | 25.9±2.10 | 0.51 | 7.42±0.62 | 0.96 |
| rs5262 (NPPC) | | | | | | | | | | | | | | |
| GG (n=674) | 129.7±0.81 | | 76.1±0.50 | | 53.6±0.64 | | 1.26±0.02 | | 179.6±3.27 | | 25.0±0.39 | | 7.47±0.11 | |
| GA (n=251) | 129.2±1.36 | | 75.2±0.83 | | 54.0±1.07 | | 1.24±0.03 | | 180.7±5.33 | | 24.9±0.63 | | 7.53±0.18 | |
| AA (n=26) | 130.2±4.36 | 0.94 | 79.3±2.69 | 0.29 | 50.8±3.46 | 0.68 | 1.18±0.11 | 0.72 | 192.9±17.2 | 0.75 | 27.8±2.04 | 0.39 | 7.57±0.60 | 0.94 |
| rs28730726 (NPRA) | | | | | | | | | | | | | | |
| GG (n=803) | 130.1±0.79 | | 75.9±0.49 | | 53.9±0.59 | | 1.25±0.02 | | 182.1±2.99 | | 24.9±0.35 | | 7.61±0.10 | |
| GC (n=143) | 131.2±1.88 | | 77.1±1.16 | | 53.4±1.40 | | 1.28±0.05 | | 171.1±7.21 | | 25.8±0.85 | | 6.92±0.25 | |
| CC (n=5) | 137.7±10.2 | 0.66 | 83.8±6.31 | 0.30 | 54.4±7.95 | 0.93 | 1.22±0.25 | 0.78 | 178.3±38.5 | 0.37 | 23.3±4.57 | 0.57 | 7.92±1.34 | 0.04 |
| rs2270915 (NPRC) | | | | | | | | | | | | | | |
| AA (n=693) | 130.4±0.80 | | 75.9±0.50 | | 54.6±0.64 | | 1.26±0.02 | | 181.7±3.23 | | 25.0±0.38 | | 7.56±0.11 | |
| AG(n=242) | 128.1±1.36 | | 76.1±0.85 | | 52.0±1.07 | | 1.22±0.04 | | 178.1±5.46 | | 25.2±0.65 | | 7.35±0.19 | |
| GG (n=16) | 127.4±5.16 | 0.32 | 78.6±3.22 | 0.70 | 49.0±4.08 | 0.06 | 1.25±0.13 | 0.58 | 154.8±19.9 | 0.36 | 22.0±2.35 | 0.43 | 7.23±0.69 | 0.59 |
| rs2292026 (NPRC) | | | | | | | | | | | | | | |
| CC (n=605) | 130.3±0.86 | | 75.6±0.53 | | 54.6±0.68 | | 1.26±0.02 | | 182.4±3.47 | | 24.9±0.41 | | 7.56±0.12 | |
| CT (n=316) | 128.9±1.20 | | 76.5±0.74 | | 52.4±0.95 | | 1.23±0.03 | | 175.0±4.73 | | 25.2±0.56 | | 7.31±0.16 | |
| TT (n=30) | 124.3±3.73 | 0.23 | 76.3±2.30 | 0.64 | 48.0±2.94 | 0.02 | 1.33±0.10 | 0.51 | 191.3±14.8 | 0.34 | 24.9±1.76 | 0.92 | 7.84±0.52 | 0.36 |
| rs11934749 (Corin) | | | | | | | | | | | | | | |
| CC (n=929) | 129.9±0.69 | | 76.0±0.43 | | 53.8±0.55 | | 1.25±0.02 | | 180.2±2.78 | | 25.0±0.33 | | 7.49±0.10 | |
| CT (n=22) | 121.9±4.71 | 0.09 | 73.7±2.91 | 0.42 | 48.3±3.73 | 0.14 | 1.34±0.12 | 0.44 | 178.3±19.3 | 0.92 | 24.0±2.29 | 0.66 | 7.93±0.67 | 0.52 |
| rs3749585 (Corin) | | | | | | | | | | | | | | |
| TT (n=329) | 129.9±1.17 | | 76.7±0.72 | | 53.2±0.93 | | 1.25±0.03 | | 179.8±4.64 | | 25.4±0.55 | | 7.43±0.16 | |
| TC (n=436) | 130.4±1.01 | | 75.7±0.63 | | 54.7±0.80 | | 1.26±0.03 | | 184.8±4.10 | | 25.2±0.49 | | 7.57±0.14 | |
| CC (n=186) | 128.0±1.55 | 0.44 | 75.5±0.96 | 0.51 | 52.5±1.23 | 0.26 | 1.24±0.04 | 0.89 | 170.6±6.21 | 0.16 | 23.8±0.74 | 0.17 | 7.45±0.22 | 0.78 |
| rs2289433 (Corin) | | | | | | | | | | | | | | |
| AA (n=470) | 130.4±0.98 | | 76.2±0.61 | | 54.2±0.78 | | 1.23±0.03 | | 178.3±3.92 | | 25.1±0.46 | | 7.41±0.14 | |
| GA (n=397) | 129.3±1.06 | | 75.8±0.65 | | 53.4±0.84 | | 1.28±0.03 | | 184.3±4.29 | | 25.1±0.51 | | 7.57±0.15 | |
| GG (n=84) | 127.6±2.28 | 0.46 | 75.1±1.40 | 0.74 | 52.4±1.80 | 0.61 | 1.25±0.06 | 0.43 | 171.4±9.27 | 0.36 | 23.6±1.10 | 0.44 | 7.67±0.32 | 0.64 |

Values are mean±SE. Systolic and diastolic blood pressure and pulse pressure were adjusted for sex, age, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs. Urinary electrolytes were measured in 847 individuals. Urinary sodium and potassium excretions were adjusted for sex, age, body mass index, serum creatinine, body surface area, and the use of antihypertensive drugs. * On the basis of analysis of variance.

**Supplementary Table 5** Blood pressure and urinary electrolytes excretions in relation to five polymorphisms of the natriuretic peptide system in the total population

| Gene and polymorphism | Systolic pressure (mmHg) | | Diastolic pressure (mmHg) | | Pulse pressure (mmHg) | | Urinary volume (l) | | Urinary sodium excretion (mmol/day) | | Urinary potassium excretion (mmol/day) | | $Na^+/K^+$ ratio | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ | mean±SE | $P^*$ |
| rs198358 (NPPA-NPPB) | | | | | | | | | | | | | | |
| AA ( n=1624) | 125.8±0.50 | | 76.2±0.29 | | 49.5±0.39 | | 1.17±0.01 | | 160.5±2.06 | | 24.5±0.29 | | 6.90±0.07 | |
| GA ( n=640) | 127.3±0.81 | | 77.3±0.46 | | 50.0±0.62 | | 1.18±0.02 | | 154.8±3.27 | | 23.5±0.46 | | 6.93±0.11 | |
| GG ( n=42) | 129.8±3.29 | 0.16 | 80.4±1.90 | 0.016 | 49.7±2.51 | 0.83 | 1.02±0.09 | 0.19 | 121.7±13.5 | 0.008 | 20.2±1.90 | 0.02 | 6.58±0.48 | 0.77 |
| rs6668352 (NPPA-NPPB) | | | | | | | | | | | | | | |
| GG ( n=1538) | 126.3±0.52 | | 76.3±0.30 | | 50.0±0.40 | | 1.18±0.01 | | 159.7±2.13 | | 24.1±0.30 | | 6.94±0.08 | |
| GA ( n=708) | 126.4±0.76 | | 77.2±0.44 | | 49.2±0.58 | | 1.16±0.02 | | 154.5±3.09 | | 23.9±0.44 | | 6.84±0.11 | |
| AA ( n=60) | 124.4±2.66 | 0.78 | 77.8±1.53 | 0.19 | 46.7±2.05 | 0.17 | 1.29±0.07 | 0.21 | 168.4±11.3 | 0.26 | 26.0±1.60 | 0.44 | 6.71±0.40 | 0.66 |
| rs3811544 (NPPC) | | | | | | | | | | | | | | |
| CC (n=2175) | 126.5±0.43 | | 76.8±0.25 | | 49.7±0.33 | | 1.13±0.01 | | 157.2±1.78 | | 24.1±0.25 | | 6.89±0.06 | |
| CT+TT (n=131) | 122.7±1.74 | 0.06 | 74.1±1.01 | 0.008 | 48.6±1.34 | 0.40 | 1.23±0.05 | 0.04 | 174.2±7.47 | 0.03 | 24.8±1.05 | 0.52 | 7.18±0.26 | 0.30 |
| rs700923 (NPRC) | | | | | | | | | | | | | | |
| AA (n=1435) | 127.0±0.53 | | 76.7±0.31 | | 50.2±0.41 | | 1.14±0.01 | | 156.0±2.17 | | 23.9±0.31 | | 6.86±0.08 | |
| GA (n=789) | 125.3±0.72 | | 76.3±0.42 | | 48.9±0.56 | | 1.14±0.02 | | 153.9±2.89 | | 23.7±0.41 | | 6.87±0.10 | |
| GG (n=82) | 123.2±2.20 | 0.06 | 77.1±1.28 | 0.65 | 46.2±1.70 | 0.02 | 1.21±0.06 | 0.48 | 160.7±8.90 | 0.71 | 24.6±1.25 | 0.77 | 6.97±0.32 | 0.94 |
| rs9662664 (NPRA) | | | | | | | | | | | | | | |
| GG (n=1029) | 125.6±0.63 | | 76.3±0.37 | | 49.3±0.49 | | 1.17±0.02 | | 160.4±2.60 | | 24.2±0.36 | | 6.96±0.09 | |
| GT (n=1021) | 126.8±0.63 | | 76.8±0.37 | | 50.1±0.49 | | 1.17±0.02 | | 156.0±2.65 | | 24.0±0.37 | | 6.88±0.09 | |
| TT (n=256) | 126.7±1.23 | 0.38 | 77.2±0.71 | 0.48 | 49.5±0.95 | 0.53 | 1.21±0.03 | 0.55 | 157.9±5.12 | 0.49 | 24.3±0.72 | 0.94 | 6.80±0.18 | 0.68 |

Values are mean±SE. Systolic and diastolic blood pressure and pulse pressure were adjusted for sex, age, body mass index, current smoking, alcohol intake, and the use of antihypertensive drugs. Urinary electrolytes were measured in 1765 individuals. Urinary sodium and potassium excretions were adjusted for sex, age, body mass index, serum creatinine, body surface area, and the use of antihypertensive drugs. * On the basis of analysis of variance.

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