Common Variation in With No-Lysine Kinase 1 (WNK1) and Blood Pressure Responses to Dietary Sodium or Potassium Interventions – Family-Based Association Study –

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**Background:** Common variations in the gene with no-lysine kinase 1 (WNK1) are associated with hypertension, but because of gene-environment interaction, it is difficult to fully identify the genetic contribution of WNK1 gene polymorphism to blood pressure (BP) variability. The aim of this study was to identify the effect of common WNK1 variants on the shift of BP during strict dietary interventions of salt or potassium intake.

**Methods and Results:** A total of 342 subjects from 126 families were selected and sequentially maintained on normal diet for 3 days at baseline, a low-salt diet for 7 days (3g/day, NaCl), then a high-salt diet for 7 days (18g/day), and high-salt diet with potassium supplementation for another 7 days (4.5g/day, KCl). Five single nucleotide polymorphisms (SNPs) were selected from the WNK1 gene. rs880054 and rs12828016 were associated with diastolic BP (DBP) response during the low- or high-sodium intervention, and rs2301880 was significantly associated with systolic BP, DBP and mean arterial pressure responses to the high-sodium intervention (all P<0.05). Unfortunately, no associations for WNK1 SNPs and the constructed haplotype blocks of WNK1 with BP responses to high-salt-and-potassium supplement intervention reached nominal statistical significance.

**Conclusions:** The WNK1 gene might be mechanistically involved in the variation in BP response to dietary sodium and potassium intake among individuals, and might contribute to the variation of this complex phenotype. (Circ J 2013; 77: 169–174)

**Key Words:** Blood pressure; Gene polymorphism; Potassium; Sodium; With no-lysine kinase 1 (WNK1)
implicated as an important modulator of salt homeostasis, regulating the balance between renal sodium reabsorption and potassium excretion. WNK1 is predominantly expressed in the distal nephron of the kidney at sites involved in regulating NaCl reabsorption and studies suggest that increased WNK1 expression would have the net result of increasing NaCl reabsorption, leading to volume expansion-induced hypertension. Mutations in WNK1 cause pseudohypaldosteronism type 2 (PHA2) – a rare autosomal dominant disorder primarily characterized by early onset hypertension and hyperkalemia. Therefore, the possibility has been proposed that genetic mutants in WNK1 affect BP variations and/or susceptibility to essential hypertension (EH). Indeed, there is accumulated evidence of associations between the common variants of WNK1 gene and BP levels. Failure to measure gene–environment interactions, however, especially regarding dietary sodium or potassium, may result in the inability to fully detect the genetic contribution to BP variability in those studies. In present study, the aim was to quantify the effect of common WNK1 variants on the shift of BP during strict dietary interventions of salt or potassium intake.

**Methods**

**Subjects**
In Northern China, a community-based BP screening was conducted among persons aged 18–60 years in the study villages to identify potential probands and their families for the study. Those with mean systolic BP (SBP) between 130 and 160 mmHg and/or diastolic BP (DBP) between 85 and 100 mmHg and no use of antihypertensive medications, and their siblings, spouses, or offspring were recruited for the dietary intervention study. Individuals who had stage 2 hypertension, secondary hypertension, a history of CVD, chronic kidney disease or diabetes, used antihypertensive medications, or were pregnant, heavy alcohol users or currently on a low-sodium diet were excluded from the dietary intervention. The institutional ethics committee of Xi’an Jiaotong University Medical School approved the study protocol, and written informed consent for the baseline observation and for the intervention program was obtained from each participant. All of the procedures were performed in accordance with institution guidelines.

**Dietary Intervention**
The protocol consisted of a series of investigations, including baseline history and physical examination (height, weight, and BP) for 3 days, 7 days on a low-salt diet (51.3 mmol or 3 g NaCl per day), 7 days on a high-salt diet (307.7 mmol or 18 g NaCl per day) and a high-salt diet with potassium supplementation (60 mmol or 4.5 g KCl per day) for another 7 days. During the entire period of the intervention, other dietary nutrient intake remained unchanged and each subject was given detailed dietary instructions to avoid table salt, cooking salt, high-sodium foods, and food rich in nitrite/nitrate for the subsequent 21 days. Total energy intake was varied according to each participant’s baseline energy intake. The study participants came to the study kitchen for their breakfast, lunch, and dinner during the entire intervention period. All foods were cooked without salt and pre-packaged salt was added to the individual study participant’s meal when it was served by the study staff onsite.

**BP Measurement and Definition of BP Response to Dietary Intervention**
Three random-zero BP measurements were obtained using a Hawksley random-zero sphygmomanometer (Hawksley & Sons, Lancing, UK; zero range 0–20 mmHg) with a 1-min interval during the 3-day baseline observation period, as well as on days 5, 6, and 7 of each intervention period. BP was measured by trained and certified observers according to a common protocol adapted from procedures recommended by the American Heart Association. BP was measured with the participant in the sitting position after 5 min of rest. In addition, participants were advised to avoid alcohol, cigarette smoking, coffee/tea, and exercise for at least 30 min prior to BP measurement. SBP and DBP were determined as the first and fifth phases of Korotkoff sounds, respectively. Mean blood pressure (MBP) was defined as: MBP = DBP + 1/3 × PP. BP at baseline and during the intervention was calculated as the mean of 9 measurements from 3 clinical visits during the 3-day baseline observation or on days 5, 6, and 7 of each intervention phase. Responses were defined as follows: BP response to low sodium=BP on low-sodium diet–BP at baseline; BP response to high sodium=BP on high-sodium diet–BP on low-sodium diet; and BP response to potassium supplement=BP on high-sodium diet with potassium supplementation–BP on high-sodium diet.

**Measurement of Sodium and Potassium in 24-h Urine**
The sodium and potassium concentrations in the urine were measured on flame photometry. The total sodium and potassium excreted in urine in 24 h were calculated by multiplying their concentration and the total volume of urine in 24 h.

**DNA Extraction and Genotyping**
Peripheral venous blood was drawn from each participant. Genomic DNA was extracted from whole blood using the Maxwell 16 DNA Purification Kit (Promega, Madison, WI, USA). The choice of single nucleotide polymorphism (SNP) was made according to the results of a prior analysis of WNK1 described. All the genotyping experiments were done by Shanghai Generay Biotech (http://www.generay.com.cn/) using ligase detection reactions (LDR). The target DNA sequences were amplified using multiplex polymerase chain reaction (PCR). After the completion of the amplification, 1 μl Proteinase K (20 mg/ml) was added, then heated at 70°C for 15 min and quenched at 94°C for 15 min. The ligation reaction for each subject was carried out in a final volume of 10 μl containing 2 μl Multi-PCR product, 1 μl probe, 0.125 μl of 40 μl/μl TaqDNA ligase (NEB, USA), 1 μl of 10×Taq DNA ligase buffer and 6 μl H2O. LDR were performed using 25 cycles of 94°C for 30 s and 55°C for 4 min. The fluorescent products of LDR were differentiated using ABI sequencer 377. Additionally, approximately 5% of the samples were randomly selected and retested by direct DNA sequencing on a 3730xl DNA analyzer (Applied Biosystems) and the results were 100% concordant.

**Statistical Analysis**
The Mendelian consistency of the SNP genotype data was assessed using PLINK and PedCheck on parental SNP data. Departure from Hardy-Weinberg equilibrium was tested with chi-square test on parental SNP data. We used Haploview (version 4.0, http://www.broad.mit.edu/mpg/haplovie.html) to estimate the extent of pairwise linkage disequilibrium between SNPs. We used Family Based Association Test (FBAT; version 2.0.2, http://www.biostat.harvard.edu/fbat/default.html) to test the association of single marker and haplotypes with adjusted phenotypes. Three genetic models (additive, dominant, and recessive) were tested. To assess the effect of genetic variants on the trait value, we used univariate FBAT test was performed for each allele and haplotype. This test provides a
**Results**

Characteristics and BP Response to Dietary Intervention

Table 1 lists the baseline characteristics and BP responses to the low-salt, high-salt and high-salt-and-potassium supplement intervention among family members. The probands had higher mean baseline SBP and DBP than their siblings, spouses, and offspring, whereas the parents had the highest baseline SBP among all of the groups. Overall, BP decreased from baseline to low-salt intervention but increased from the low-sodium to high-sodium intervention. For both low-salt, high-salt, and high-salt-and-potassium supplement interventions, the probands had greater changes in mean arterial pressure (MAP) than their siblings, spouses, and offspring.

**Influence of Dietary Intervention on Urinary Sodium and Potassium Excretion**

At baseline, the high sodium and low potassium excretion suggested that the dietary pattern of Northern Chinese people is characterized by high sodium intake and insufficient intake of potassium, in line with our previous survey. To ensure compliance with the intervention program, the total volume of sodium in the urine was calculated at the end of each diet period. As shown in Table 2, it was remarkably higher during the high-salt diet than during the low-salt diet. Meanwhile, potassium...
supplement increased not only urinary potassium excretion but also sodium excretion slightly. The result confirmed the dietary intervention was successful.

### Allele Frequencies and Hardy-Weinberg Equilibrium Test

Figure and Table 3 lists the genomic location, allele frequency, and Hardy-Weinberg tests for the 5 WNK1 SNPs analyzed. None of the SNPs deviated statistically significantly from Hardy-Weinberg equilibrium.

**Table 3. WNK1 SNPs Genotyped**

| WNK1 SNP     | WNK1 Position | Alleles | Minor allele frequency | Hardy-Weinberg test† | Pearson's χ² | P-value |
|--------------|---------------|---------|------------------------|----------------------|-------------|---------|
| rs880054     | Intron 10     | A/G     | 0.27                   | 0.72                 | 0.396       |
| rs12828016   | Exon 21       | Met/Ile | 0.25                   | 0.022                | 0.83        |
| rs956868     | Exon 13       | Pro/Thr | 0.21                   | 1.83                 | 0.176       |
| rs2301880    | Intron 23     | C/T     | 0.21                   | 0.029                | 0.865       |
| rs765250     | Intron 1      | A/G     | 0.12                   | 0.004                | 0.949       |

†Parents only (parental generation).
SNP, single nucleotide polymorphism; WNK1, with no-lysine kinase 1.

**Table 4. SNPs Significantly Associated With BP Response to Dietary Intervention**

| SNP            | Allele | SBP response | DBP response | MAP response |
|----------------|--------|--------------|--------------|--------------|
|                |        | z            | P-value      | z            | P-value      | z            | P-value      |
| Low-sodium intervention |       |              |              |              |              |              |              |
| rs880054       | A      | –0.982       | 0.325        | 2.261        | 0.023        | –1.873       | 0.061        |
| rs12828016     | G      | –0.935       | 0.357        | 2.506        | 0.012        | –1.936       | 0.052        |
| rs956868       | A      | –1.195       | 0.232        | –1.215       | 0.224        | 0.726        | 0.467        |
| rs2301880      | C      | –1.142       | 0.253        | 0.473        | 0.636        | 1.286        | 0.198        |
| rs765250       | A      | 0.217        | 0.828        | 0.240        | 0.810        | 0.054        | 0.957        |
| High-sodium intervention |       |              |              |              |              |              |              |
| rs880054       | A      | 0.463        | 0.643        | 2.063        | 0.039        | 1.641        | 0.100        |
| rs12828016     | G      | 0.527        | 0.598        | 2.516        | 0.011        | 2.013        | 0.044        |
| rs956868       | A      | 0.753        | 0.451        | –1.931       | 0.053        | –1.548       | 0.121        |
| rs2301880      | C      | 1.958        | 0.046        | 2.228        | 0.025        | 2.195        | 0.028        |
| rs765250       | A      | –0.067       | 0.946        | –0.329       | 0.741        | –0.256       | 0.798        |
| High-salt-and-potassium supplement intervention |       |              |              |              |              |              |              |
| rs880054       | A      | –0.635       | 0.525        | –0.558       | 0.576        | –0.597       | 0.550        |
| rs12828016     | G      | –0.527       | 0.598        | –0.380       | 0.704        | –0.858       | 0.390        |
| rs956868       | A      | –1.120       | 0.262        | –1.098       | 0.272        | –0.914       | 0.360        |
| rs2301880      | C      | –0.805       | 0.420        | –0.646       | 0.518        | –0.713       | 0.475        |
| rs765250       | A      | –0.490       | 0.623        | –0.450       | 0.652        | –0.129       | 0.897        |

P-values are corrected for multiple testing (FDR<0.05).
DBP, diastolic BP; FBAT, family-based association test; FDR, false discovery rate; MAP, mean arterial pressure; SBP, systolic BP; z, test statistic for FBAT. Other abbreviations as in Tables 1,3.
**WNK1 and BP Response to Dietary Intervention**

As shown in Table 4, FBAT identified significant associations for rs880054 and rs12828016 with DBP response during the low-sodium or high-sodium intervention. SNP rs2301880 was significantly associated with SBP, DBP and MAP responses to high-sodium intervention (all P<0.05). Unfortunately no associations for WNK1 SNPs with BP responses to high-salt-and-potassium supplement reached nominal statistical significance. Furthermore, we also constructed haplotype blocks of WNK1 SNPs using Haploview, and the 5 SNPs were located in 1 haploblock. No haplotypes, however, were associated with SBP, DBP or MAP responses to dietary intervention (Table 5).

**Discussion**

In the present study we identified several SNPs in the WNK1 gene, such as rs880054, rs12828016, rs2301880, which were nominally significantly associated with BP responses to dietary sodium intervention. The results indicated that the WNK1 gene might be mechanistically involved in BP salt sensitivity and that these genetic variants might contribute to the variation of this complex phenotype.

The present study had several important strengths. First, the subjects were recruited from several rural neighboring communities that were similar with respect to lifestyle and environmental risk factors, including diet and physical activity. Furthermore, this study was based on the family pedigree. Thus, the confounding of genetic associations due to these factors should be minimized. In addition, BP response to sodium or potassium supplementation was measured under a controlled dietary salt intake or potassium supplementation, which provides a good opportunity to identify dedication of gene polymorphism in the heterogeneity of these responses to BP. At the same time, BP response to dietary sodium or potassium intervention was measured as a continuous variable, rather than being categorized with a cut-point (such that individuals would have been divided into salt- or potassium-sensitive and salt- or potassium-resistant groups), to improve the sensitivity of the detection of the small effect of the gene polymorphisms. Last, participation in the dietary interventions was high, and compliance with the study interventions, as assessed on urinary excretion of sodium and potassium during each intervention period, was excellent.

WNK1 is thought to be a key factor in maintaining sodium and potassium homeostasis in the kidney, and consequently BP regulation. In the kidney, WNK1 is expressed in 2 splicing variants, namely KS-WNK1 and L-WNK1.24,25 L-WNK1 activates the epithelial Na channel (ENaC) and Na+/Cl– cotransporter (NCC), thus motivating sodium reabsorption. KS-WNK1 inhibits WNK1 kinase activity and inhibits its effects on the NCC, presumably through a dominant-negative mechanism. In addition, L-WNK1 also increases NCC activity by antagonizing WNK4-mediated inhibition of this transporter.16–18,26 In addition, WNK1 haploinsufficiency in mice, shown by a 50% reduction in WNK1 RNA expression, has modestly reduced BP (approximately 12-mmHg decrease in MAP).27 It also confirmed that gain of WNK1 function causes BP elevation. Moreover, WNK1 is also a regulator of potassium homeostasis. L-WNK1 reduces potassium excretion by inhibiting renal outer medullary potassium channel (ROMK), whereas KS-WNK1 antagonizes L-WNK1 with respect to its effects on ROMK. Thus a positive ratio of L-WNK1 to KS-WNK1 decreases the rate of K+ secretion via ROMK. Interestingly, recent animal studies have shown that high potassium intake could increase the expression of the mRNA and protein of KS-WNK1 in rats.28 These findings indicate that WNK1 may be a stimulator of sodium reabsorption and potassium excretion, and common variation in WNK1 may lead to the heterogeneity in BP responses to dietary sodium or potassium interventions.

There are several studies indicating that the common variations in WNK1 are associated with hypertension and sodium or potassium homeostasis. Newhouse et al tested for association among 19 WNK1 SNPs and EH in 712 severely hyperten-

| Table 5. Haplotypes and BP Response to Dietary Intervention |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| **SNP**         | **SBP response**| **DBP response**| **MAP response**|
| **z**            | **P-value**     | **z**            | **P-value**     | **z**            | **P-value**     |
| **Low-sodium intervention** |                |                  |                  |                  |                  |
| Haplotype 1      | 0.709           | 0.478            | –0.304           | 0.761            | 0.089            | 0.929           |
| Haplotype 2      | –0.966          | 0.334            | 0.047            | 0.963            | –0.342           | 0.732           |
| Haplotype 3      | 0.027           | 0.979            | –0.567           | 0.571            | –0.338           | 0.736           |
| Haplotype 4      | 0.083           | 0.933            | 0.985            | 0.325            | 0.653            | 0.514           |
| Haplotype 5      | 0.743           | 0.458            | 1.108            | 0.268            | 1.038            | 0.299           |
| **High-sodium intervention** |                |                  |                  |                  |                  |
| Haplotype 1      | 1.36            | 0.174            | 0.11             | 0.912            | 0.622            | 0.534           |
| Haplotype 2      | –1.145          | 0.252            | –0.246           | 0.806            | –0.602           | 0.547           |
| Haplotype 3      | –0.185          | 0.853            | 0.406            | 0.685            | 0.179            | 0.858           |
| Haplotype 4      | 0.464           | 0.643            | 0.171            | 0.865            | 0.294            | 0.769           |
| Haplotype 5      | –0.58           | 0.562            | –0.172           | 0.863            | –0.339           | 0.734           |
| **High-salt-and-potassium supplement intervention** |                |                  |                  |                  |                  |
| Haplotype 1      | 1.916           | 0.055            | 1.597            | 0.110            | 1.852            | 0.064           |
| Haplotype 2      | –1.078          | 0.281            | –1.564           | 0.118            | –1.5              | 0.134           |
| Haplotype 3      | –0.714          | 0.475            | 0.379            | 0.705            | –0.069           | 0.945           |
| Haplotype 4      | –0.242          | 0.809            | –0.518           | 0.605            | –0.445           | 0.857           |
| Haplotype 5      | 0.46            | 0.646            | –0.784           | 0.433            | –0.35             | 0.726           |

Abbreviations as in Tables 1,3,4.
sive families, and observed suggestive evidence for an association between variants of WNK1 and severity of hypertension. Moreover, Tobi et al also found that rs880054 in WNK1 contributes to BP variation in a population-based sample of 996 subjects from 250 white European families. Recently, Osada et al reported that not only were rs880054, rs956868, and rs12828016 in the WNK1 gene associated with BP variations in the general Japanese population, but also the constructed haplotypes were associated with Na/K intake ratio, which hints that the part of the variation in BP response to dietary sodium and potassium intake among individuals can be explained by variations in the WNK1 gene. In addition, Manunta et al showed that the WNK1 genotypes produced greater urinary Na and K excretion under acute Na load than WNK1 G carriers in rs880054. In the current study, WNK1 gene variation was nominally significantly associated with BP response to dietary sodium intervention, but not to dietary potassium intervention. Failure to find an effect of the 5 gene polymorphisms on BP response to dietary potassium intervention could be due to genetic heterogeneity across populations, small effect size or low power. We also found no association of haplotype with BP responses to dietary sodium or potassium intervention. WNK1 is a relatively large gene, and although we assumed that the 5 SNPs lie in a single block, it is also possible that the SNPs could span 1 haplotype block, which would lead to the discrepancy of association between gene polymorphism and haplotype.

The present study has some other limitations that should be addressed. Because all of the subjects were recruited from the Northern Chinese population, the present results will require replication in other cohorts to determine generalizability to other ethnicities and to populations with different dietary habits. Furthermore, further studies to identify the causal polymorphism loci along with their functions are also warranted.

**Conclusion**

rs880054, rs12828016, and rs2301880 in the WNK1 gene were significantly associated with BP response to dietary sodium intervention, and these findings may contribute to a better understanding of the genetic mechanisms underlying BP regulation and may have potential clinical and public health implications.

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