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ORIGINAL ARTICLE

Variations in the *WNK1* gene modulates the effect of dietary intake of sodium and potassium on blood pressure determination

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WNK lysine-deficient protein kinase 1 (WNK1) is a member of the WNK family of serine/threonine kinases with no lysine (K), and these kinases have been implicated as important modulators of salt homeostasis in the kidney. It is well known that high dietary sodium and low dietary potassium have been implicated in the etiology of increased blood pressure. However, the blood pressure response to dietary sodium and potassium intake varies considerably among individuals. In this study, we have detected that the haplotypes of the *WNK1* gene are associated with blood pressure variations in the general Japanese population. In addition, we investigated the interactions between the haplotypes of the *WNK1* gene and dietary sodium and potassium intake for determining inter-individual variations in blood pressure. Our data support the hypothesis that part of the variation in blood pressure response to dietary sodium and potassium intake among individuals can be explained by variations in the *WNK1* gene.
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Keywords: blood pressure; haplotype; potassium; sodium; WNK1

INTRODUCTION

Essential hypertension is one of the most common diseases of the modern world. High blood pressure or hypertension contributes to morbidity and mortality from stroke, coronary heart disease and end-stage renal disease.[1] Both family and epidemiological studies have suggested that 20–50% of blood pressure variation that exists within a population is of genetic origin;[2,3] however, the genes responsible for this variation in blood pressure have not been fully elucidated.

Blood pressure is maintained by a complex network, including renal, neuronal, endocrine and vascular mechanisms. Therefore, blood pressure is determined by complex interactions among many different genes, and is strongly influenced by environmental factors. Clinical and epidemiological studies have shown that dietary sodium intake is positively associated, and potassium intake is inversely associated, with blood pressure.[4,5] In addition, the dietary sodium to potassium (Na/K) intake ratio correlates positively with blood pressure more strongly than either sodium or potassium intake alone.[4] Therefore, the effects of sodium and potassium intake on blood pressure are most likely to be biologically interdependent. Furthermore, moderate sodium reduction or potassium supplementation is significantly associated with reduced blood pressure. However, the blood pressure response to dietary sodium and potassium intake seems to vary considerably among individuals.[6]

The WNK lysine-deficient protein kinase 1 (*WNK1*) and *WNK4* genes are expressed in the distal nephron, specifically in the distal convoluted tubule and the connecting tubule and collecting duct.[7] WNK1 and WNK4 have been implicated as important modulators of salt homeostasis, regulating the balance between renal sodium reabsorption and potassium excretion.[8–10] Mutations in *WNK1* or *WNK4* genes cause a familial disorder, pseudohypoaldosteronism type II, which is a rare, autosomal dominant disease characterized by hypertension and elevated serum potassium levels with normal renal function.[7] Therefore, the possibility has been proposed that common genetic variants in both WNK1 and WNK4 affect blood pressure variations and/or susceptibility to essential hypertension.[11,12] Indeed, several studies have indicated associations between the common variants of *WNK1* and *WNK4* genes and blood pressure levels.[11,13–16] However, the functional common variants that directly affect the activity of WNK1 or WNK4 have not been identified.

WNK1 is expressed in multiple splicing variants. At least two transcripts, a kidney-specific short form of WNK1 (KS-WNK1) and a more ubiquitous long form (L-WNK1), have been produced by alternative splicing of the *WNK1* gene in the kidney.[17,18] Recent studies have suggested that the ratio of L-WNK1 to KS-WNK1 is important for the regulation of renal sodium reabsorption and potassium secretion.[12] Furthermore, it has been proposed that the

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molecular switch for the splicing of L-WNK1 and KS-WNK1 is affected by the amount of dietary potassium intake.[19–21] In this study, we investigated whether the common variations in the *WNK1* gene are potential contributors to individual variations in blood pressure. Furthermore, we analyzed the interactions between the common *WNK1* variants and dietary potassium and sodium intake for determining inter-individual variations in blood pressure.

MATERIALS AND METHODS

Participants

The participants were 691 randomly selected, apparently healthy Japanese men (mean age ± s.d., 53.7 ± 5.1 years; age range, 45–65 years), who visited a medical center for routine medical checkups. All participants provided written informed consent, and the study was approved by the Ethics Committee of the University of Shizuoka. Men taking antihypertensive drugs were excluded from the study. After overnight fasting, blood samples were collected from each participant. Resting blood pressure level was measured using a mercury sphygmomanometer in participants who were in the sitting position after at least 10 min of rest. Of the 691 men, 97 were diagnosed with hypertension according to the standard WHO (World Health Organization) criteria (systolic blood pressure (SBS) $\geqslant$140 mm Hg and/or diastolic blood pressure (DBP) $\geqslant$90 mm Hg) by a physician. The clinical characteristics of the participants are shown in Table 1.

Sodium and potassium intake assessment

The levels of dietary sodium, potassium and alcohol intake were estimated using a brief-type self-administered diet history questionnaire (BDHQ).[22,23] The BDHQ was developed on the basis of the self-administered diet history questionnaire (DHQ), which has been validated using three different standard methods for dietary assessment.[24] The BDHQ and DHQ were designed to obtain dietary habits for the previous month for total energy and 38 nutrients including sodium and potassium.[22–24] Dietary data were available for 615 participants (89.0%) in this study.

Table 1 Characteristics of the study participants

	n=*691 (615)*
Age (years)	53.7 ± 5.1
SBP (mm Hg)	120.6 ± 15.6
DBP (mm Hg)	76.7 ± 11.6
Body mass index (kg m^{-2})	23.5 ± 2.8
Total cholesterol (mg per 100 ml)	211.4 ± 31.8
HDL cholesterol (mg per 100 ml)	56.7 ± 16.3
Triglyceride (mg per 100 ml)	140.5 ± 104.3
Diabetes mellitus (%)	2.6
Current smoking (%)	42.7
Total energy intake (kcal per day)	2085 ± 570
Sodium intake (mg per day)	4522 ± 1352
Potassium intake (mg per day)	2305 ± 795
Sodium to potassium intake ratio	2.03 ± 0.41
Alcohol intake (g per day)	28.0 ± 32.0

Abbreviations: DBP, diastolic blood pressure; HDL, high-density lipoprotein; SBP, systolic blood pressure.
Data are expressed as mean ± s.d. or percentages.
Number of participants whose dietary intake data were available is in parenthesis.

DNA analysis

Genomic DNA was isolated from peripheral blood leukocytes using the phenol extraction method. We have determined the genotypes of five single-nucleotide polymorphisms (SNPs) of the *WNK1* gene (rs2286007, rs880054, rs956868, rs12828016 and rs2255390) by PCR–restriction fragment length polymorphism analysis. We have selected these SNPs on the basis of the public NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/). At first, we have analyzed the heterozygosity and genotyping success rate for 12 SNPs (rs11554421, rs2369402, rs2240282, rs2286006, rs2286007, rs880054, rs9804992, rs16928108, rs956868, rs12301299, rs12828016 and rs2255390) in a pilot study comprising 96 participants, and selected 3 non-synonymous SNPs (rs2286007, rs956868 and rs12828016) with a relatively high heterozygosity (minor allele frequency (MAF) > 0.10) and two intronic SNPs (rs880054 and rs2255390) with high heterozygosity (MAF > 0.45). The genotyping success rate for each of these five SNPs is >98%. The genomic structure of the *WNK1* gene and locations of these five SNPs are shown in Figure 1.

Statistical analysis

The relationships between blood pressure levels and each genotype or haplotype of the *WNK1* gene were analyzed by multiple linear regression analysis incorporating age, body mass index (BMI), smoking and alcohol consumption as covariates. Statistical analyses were carried out using the JMP software package (SAS Institute, Cary, NC, USA). $P<0.05$ was considered statistically significant. No adjustment for multiple testing was made. The coefficients of linkage disequilibrium (LD) ($|D'|$ and r^2) were calculated using the SNPAlyze program (Dynacom, Yokohama, Japan). The haplotypes and their frequencies were estimated by the maximum-likelihood method with an EM-based algorithm using the SNPAlyze program.

RESULTS

We analyzed the relationships between the genotypes of five SNPs (rs2286007, rs880054, rs956868, rs12828016 and rs2255390) in the *WNK1* gene and blood pressure levels. Statistically significant associations were observed between the genotypes of three SNPs (rs880054, rs956868 and rs12828016) and SBP and/or DBP levels in the multiple linear regression analysis adjusted for age, BMI, smoking and alcohol consumption as covariates. The A allele (rs880054, A > G polymorphism in intron 10), the Thr allele (rs956868, Pro1056Thr polymorphism in exon 13) and the Met allele (rs12828016, Met1808Ile polymorphism in exon 21) were associated with increased blood pressure with gene dosage effects. On the other hand, the genotypes of the remaining two SNPs (rs2286007 and rs2255390) were not associated with blood pressure levels (Table 2).

These five SNPs were in LD with each other ($|D'|>0.85|$), in particular the genotypes of the A > G polymorphism in intron 10 (rs880054) and the Met1808Ile polymorphism (rs12828016) were

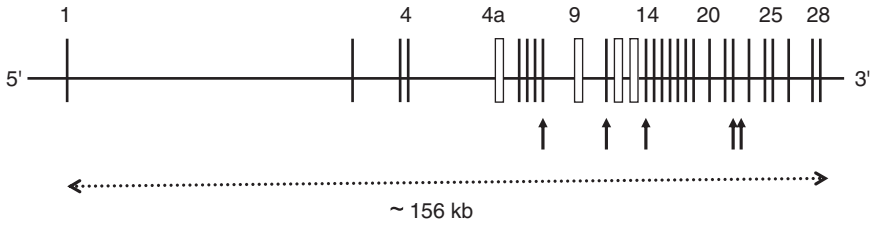

Figure 1 Genomic structure of the *WNK1* gene and location of the genotyped single-nucleotide polymorphisms (SNPs). Exons are indicated by the vertical bars and alternatively spliced exons by the white boxes. The locations of the five genotyped SNPs are indicated by arrows.

identical in almost all participants (98.0%). The pairwise LD values of $|D'|$ and r^2 are shown in Table 3. There are four common haplotypes with frequencies of >5% that account for 97% of all chromosomes in our participants (Table 4).

The SBP level in carriers of haplotype 3 (Thr-A-Thr-Met-G) was higher than that in noncarriers of this haplotype (P=0.025) (Table 5). On the other hand, the SBP and DBP levels of carriers of haplotype 2 (Thr-G-Pro-Ile-G) were lower than those in noncarriers of this haplotype (P=0.017, P=0.0083, respectively) (Table 5). These data indicate that haplotype 3 is associated with increased blood pressure levels, whereas haplotype 2 is associated with decreased blood pressure levels.

Next, to examine the interaction between the haplotypes of the *WNK1* gene and dietary sodium and potassium intake for determining blood pressure levels, we classified the participants into two groups according to population median for the Na/K intake ratio. As expected, of the total participants, the SBP and DBP levels in the group with a high Na/K intake ratio were higher than those of the group with a low Na/K intake ratio (P=0.0085, P=0.013, respectively) (Table 6). These significant differences in blood pressure levels caused by the dietary Na/K intake ratio were observed in carriers of both haplotype 1 (Thr-A-Pro-Met-A) and haplotype 2. Among the carriers of haplotype 1 and haplotype 2, the SBP or DBP levels in the group with a high Na/K intake ratio were significantly higher than those in the group with a low Na/K intake ratio (P=0.019, P=0.025, respectively) (Table 6). On the other hand, the blood pressure levels in carriers with haplotype 3 were similar among both the high and low Na/K intake ratio groups. These findings suggest that the genetic differences in the *WNK1* gene influence the variations in blood pressure response to dietary sodium and potassium intake.

Table 2 Relations between genotypes of five SNPs in *WNK1* and the SBP and DBP levels

db SNP	*Region*	*Genotype*	n	*SBP (mm Hg)*	*DBP (mm Hg)*
rs2286007	Exon 8	Thr/Thr	579	120.6 ± 16.0	76.6 ± 11.8
(Thr665Ile)		Thr/Ile	100	120.9 ± 14.0	78.0 ± 11.1
		Ile/Ile	10	112.2 ± 14.4	69.8 ± 12.4
				P=0.13	P=0.16
rs880054	Intron 10	AA	279	122.6 ± 17.3	78.0 ± 12.1
(A>G)		AG	319	119.8 ± 14.4	76.3 ± 11.5
		GG	88	116.6 ± 13.8	74.1 ± 10.4
				P=0.0088	**P=0.045**
rs956868	Exon 13	Pro/Pro	505	119.6 ± 15.7	76.1 ± 11.5
(Pro1056Thr)		Pro/Thr	171	122.8 ± 15.5	78.3 ± 12.3
		Thr/Thr	15	125.3 ± 14.0	78.3 ± 9.8
				P=0.032	P=0.20
rs12828016	Exon 21	Met/Met	283	122.7 ± 17.1	78.1 ± 12.0
(Met1808Ile)		Met/Ile	312	119.8 ± 14.3	76.3 ± 11.5
		Ile/Ile	92	117.1 ± 13.9	74.3 ± 10.7
				P=0.0096	**P=0.043**
rs2255390	Intron 22	GG	184	119.5 ± 14.0	75.8 ± 11.6
(G>A)		GA	331	120.6 ± 15.4	76.9 ± 11.3
		AA	173	121.3 ± 17.8	77.3 ± 12.6
				P=0.47	P=0.44

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure; SNP, single-nucleotide polymorphism.
Values are shown as mean ± s.d.
P-values were calculated by multiple linear regression analyses incorporating age, BMI, current smoking and alcohol intake as covariates. Statistically significant *P* values are indicated in bold.

DISCUSSION

Although epidemiological and genetic studies have shown a high heritability of blood pressure variation, identification of the genes responsible for essential hypertension has been very difficult owing to the complexity and heterogeneity of the trait. The regulation of net renal sodium reabsorption and potassium excretion in the kidney is a major determinant of blood pressure.[3,25] Furthermore, the most widely studied environmental aspect of hypertension is the relationship between sodium and potassium intake.[4] However, the blood pressure response to dietary sodium and potassium intake is believed to vary considerably among individuals. Genetic factors may also have

Table 3 The pairwise linkage disequilibrium (LD) values of $|D'|$ (upper) and r^2 (lower)

	$\|D'\|$				
r^2	*rs2286007 (Thr665Ile)*	*rs880054 (A>G)*	*rs956868 (Pro1056Thr)*	*rs12828016 (Met1808Ile)*	*rs2255390 (G>A)*
rs2286007 (Thr665Ile)		0.87	1	0.89	0.94
rs880054 (A>G)	0.13		1	0.99	0.98
rs956868 (Pro1056Thr)	0.016	0.095		1	0.97
rs12828016 (Met1808Ile)	0.14	0.96	0.095		0.98
rs2255390 (G>A)	0.083	0.54	0.16	0.53	

$|D'|$, standardized disequilibrium coefficient.
r^2, squared correlation coefficient.

Table 4 Estimated *WNK1* haplotypes and frequencies in the general Japanese population

	rs2286007 Thr665Ile	*rs880054 A>G*	*rs956868 Pro1056Thr*	*rs12828016 Met1808Ile*	*rs2255390 G>A*	*Frequency (%)*
Haplotype 1	**Thr**	**A**	**Pro**	**Met**	A	48.1
Haplotype 2	**Thr**	G	**Pro**	Ile	**G**	28.3
Haplotype 3	**Thr**	**A**	Thr	**Met**	**G**	13.8
Haplotype 4	Ile	G	**Pro**	Ile	**G**	7.8

Major alleles are indicated in bold.

Table 5 Comparison of blood pressure levels between carriers and non-carriers of each haplotype

		N	*SBP (mm Hg)*	*DBP (mm Hg)*
Haplotype 1	(+)	488	120.9 ± 16.2	77.1 ± 11.7
	(−)	180	119.3 ± 14.2	75.6 ± 11.7
			P=0.23	*P*=0.19
Haplotype 2	(+)	312	118.8 ± 14.5	75.3 ± 11.1
	(−)	356	122.0 ± 16.5	78.0 ± 12.0
			***P*=0.017**	***P*=0.0083**
Haplotype 3	(+)	173	123.3 ± 15.5	78.3 ± 12.1
	(−)	495	119.5 ± 15.6	76.1 ± 11.5
			***P*=0.025**	*P*=0.18
Haplotype 4	(+)	99	120.3 ± 13.9	77.5 ± 11.3
	(−)	569	120.5 ± 16.0	76.5 ± 11.8
			P=0.92	*P*=0.32

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.
P-values were calculated by multiple linear regression analyses incorporating age, BMI, smoking and alcohol intake as covariates. Statistically significant *P* values are indicated in bold.

Table 6 Interactions between *WNK1* haplotypes and sodium to potassium (Na/K) intake ratio

		Low Na/K intake group		*High Na/K intake group*		
		n	*SBP (mm Hg)*	n	*SBP (mm Hg)*	P-*value*
Total participants		309	119.0 ± 15.7	306	122.0 ± 15.6	**0.0085**
Haplotype 1	(+)	220	119.2 ± 16.5	217	122.7 ± 15.8	**0.019**
	(−)	80	118.1 ± 13.2	73	119.5 ± 15.0	0.32
Haplotype 2	(+)	141	117.2 ± 14.0	133	120.4 ± 15.1	0.057
	(−)	159	120.5 ± 17.0	157	123.2 ± 16.0	0.07
Haplotype 3	(+)	71	122.6 ± 14.2	79	123.3 ± 16.2	0.65
	(−)	229	117.8 ± 16.0	211	121.4 ± 15.4	0.017
Haplotype 4	(+)	41	118.5 ± 15.0	41	121.0 ± 12.7	0.48
	(−)	259	119.0 ± 15.9	249	122.1 ± 16.1	0.018
		n	*DBP (mm Hg)*	n	*DBP (mm Hg)*	P-*value*
Total participants		309	75.5 ± 12.0	306	77.9 ± 10.9	**0.013**
Haplotype 1	(+)	220	75.9 ± 12.1	217	78.3 ± 10.9	0.053
	(−)	80	74.4 ± 11.9	73	76.2 ± 11.0	0.26
Haplotype 2	(+)	141	73.8 ± 11.5	133	76.7 ± 10.6	**0.025**
	(−)	159	77.0 ± 12.3	157	78.6 ± 11.1	0.33
Haplotype 3	(+)	71	78.2 ± 12.3	79	77.5 ± 11.0	0.46
	(−)	229	74.7 ± 11.8	211	77.9 ± 10.9	0.0052
Haplotype 4	(+)	41	77.7 ± 12.5	41	76.7 ± 9.2	0.55
	(−)	259	75.2 ± 11.9	249	77.9 ± 11.2	0.0094

Abbreviations: BMI, body mass index; DBP, diastolic blood pressure; SBP, systolic blood pressure.
P-vales were calculated by multiple linear regression analyses incorporating age, BMI, current smoking and alcohol intake as covariates. Statistically significant *P* values are indicated in bold.

an important role not only in determining blood pressure levels but also in determining the blood pressure responses to dietary sodium and potassium intake.[6]

The WNK kinases and several ion transport systems in the kidney work together for maintaining sodium and potassium homeostasis, and consequently blood pressure regulation.[8,9,12,26] WNK1 is expressed in two splicing variants in the kidney, namely KS-WNK1 and L-WNK1.[17,18] They have remarkably different effects; KS-WNK1 antagonizes the ability of L-WNK1 to inhibit renal outer medullary K^+ (ROMK) and to activate the epithelial Na channel (ENaC) and Na^+Cl^- cotransporter (NCC). Therefore, a decrease in the ratio of L-WNK1 to KS-WNK1 enhances potassium secretion through ROMK and decreases sodium reabsorption through ENaC and NCC.[8,12] Therefore, the ratio of L-WNK1 to KS-WNK1 is very important for the regulation of renal potassium secretion and sodium reabsorption. Recent studies have indicated that the expression of the mRNA and protein of KS-WNK1 is significantly increased by a high potassium diet compared with a low potassium diet in rats.[19,20] These findings indicate the possibility that variations in dietary potassium intake cause reciprocal changes in the ratio of L-WNK1 to KS-WNK1 in human kidneys, and consequently in the regulation of renal potassium secretion and sodium reabsorption.

In this study, we have detected that the haplotypes of the *WNK1* gene that are associated with individual variations of blood pressure in the general Japanese population, and that the individual blood pressure response to dietary sodium and potassium intake differs among the *WNK1* haplotypes. We speculate that the L-WNK1 and KS-WNK1 expression ratio in the kidney is affected not only by dietary potassium intake but also by variations in the *WNK1* gene. The possibility exists that KS-WNK1 expression is not increased in carriers of haplotype 3 when their potassium intake is increased. On the other hand, for the carriers of haplotype 1 or haplotype 2, the expression ratio of L-WNK1 to KS-WNK1 in the kidney may be modulated by the amount of dietary sodium and potassium intake.

The possibility exists that some of the individual differences in blood pressure response to dietary sodium and potassium intake are represented by variations in the *WNK1* gene. However, at present, we have no direct evidence that such variations in the *WNK1* gene cause alterations in the expression ratio of L-WNK1 and KS-WNK1 and/or the in the activity of L-WNK1 *in vivo*. Further *in vivo* and *in vitro* studies are required to determine the functional changes in WNK1 and the ion transport systems in the kidney.

We found that an appropriate Na/K intake ratio is effective in decreasing blood pressure levels for carriers of haplotype 1 or haplotype 2 in the WNK1 gene; however, this is not effective for carriers of the haplotype 3. It will be important to gather scientific evidence of any interactions or otherwise between genetic and modifiable environmental factors such as dietary nutrient intake to establish a preventive method for common diseases, such as hypertension. If such interactions are detected, then modification of such dietary exposure may be suitable for certain population subgroups with important health consequences.

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