Yoshihiro Kokubo · Kei Kamide · Nozomu Inamoto
Chihiro Tanaka · Mariko Banno · Shin Takiuchi
Yuhei Kawano · Hitonobu Tomoike · Toshiyuki Miyata

Identification of 108 SNPs in *TSC*, *WNK1*, and *WNK4* and their association with hypertension in a Japanese general population

Received: 31 May 2004 / Accepted: 22 June 2004 / Published online: 11 August 2004
© The Japan Society of Human Genetics and Springer-Verlag 2004

Abstract The deletion of thiazide-sensitive Na–Cl cotransporter (*TSC*, *SLC12A3*) causes Gitelman's syndrome characterized by low blood pressure, while deletions of the *WNK1* (*PRKWINK1*) and *WNK4* (*PRKWINK4*) genes cause familial hypertension known as pseudohypoaldosteronism type II. Recent studies have revealed that cell surface expression of *TSC* is regulated by *WNK1* and *WNK4*. We hypothesized that molecular variations in *TSC*, *WNK1*, and *WNK4* could lead to an increased morbidity of hypertension. We identified 52, 35, and 21 polymorphisms in Japanese hypertensives by sequencing the entire coding regions of *TSC*, *WNK1* and *WNK4*, respectively. Twenty-one representative polymorphisms were genotyped in 1,818 Japanese individuals (771 subjects with hypertension and 1,047 controls) randomly sampled in Suita city. The results indicated that the systolic blood pressure in men with the CT+TT genotype in *WNK4* C14717T was 3.1 mmHg higher than those with the CC genotype ($p=0.042$) after adjustment with confounding factors such as age, BMI, hyperlipidemia, diabetes mellitus, antihypertensive drug use, smoking, and drinking. Multivariate logistic regression analysis (with adjustment for the same parameters) in men revealed that the odds ratio for the presence of hypertension of the CT+TT genotype in C14717T to the CC genotype was

1.62 ($p=0.010$, 95% confidence interval, 1.12–2.33). Association of *TSC* and *WNK1* with hypertension was not observed. In conclusion, our study suggests the possible involvement of *WNK4* in essential hypertension in a Japanese general population.

Keywords *WNK1* · *WNK4* · Thiazide-sensitive Na–Cl cotransporter · Gene variants · Hypertension

Introduction

Several molecular variants of the thiazide-sensitive Na–Cl cotransporter (*TSC*, *SLC12A3*) relate to Gitelman's syndrome characterized by their low blood pressure (BP) sodium wasting, secondary hyperaldosteronism, hypokalemia, alkalosis, hypomagnesemia, and hypocalciuria (Mastroianni et al. 1996; Simon et al. 1996; Takeuchi et al. 1996). This syndrome is known to be heritable as autosomal recessive, and the mutations identified in *TSC* may reduce the capacity of the TSC to reabsorb salt in the distal tubules where the cotransporter is regionally expressed (Mastroianni et al. 1996). On the contrary, mutations in the *WNK1* (*PRKWINK1*) and *WNK4* (*PRKWINK4*) genes relate to familial hypertension known as pseudohypoaldosteronism type II (Wilson et al. 2001), associated with hyperkalemia (despite normal renal glomerular filtration) and renal tubular acidosis caused by impaired renal K^+ and H^+ excretion. This autosomal dominant disease includes several types of mutations; a large deletion in intron 1 of *WNK1*, missense mutations in the highly conservative regions of *WNK4* (Wilson et al. 2001). Mutations identified in *WNK4* so far were all accompanied by charge changes, assuming modification of the protein function.

Recent expression studies have revealed a close link between *TSC* and *WNK* family proteins. Coexpression of *TSC* with *WNK4* leads to a significant decrease in thiazide-sensitive sodium uptake (Choate et al. 2003; Wilson et al. 2003). *WNK4* was shown consistently to

Y. Kokubo (✉) · N. Inamoto · H. Tomoike
Division of Preventive Cardiology,
National Cardiovascular Center, 5-7-1 Fujishirodai,
Suita 565-8565, Japan
E-mail: ykokubo@hsp.ncvc.go.jp
Tel.: +81-6-68335012
Fax: +81-6-68335300

K. Kamide · S. Takiuchi · Y. Kawano
Division of Hypertension and Nephrology,
National Cardiovascular Center, Suita,
Japan

C. Tanaka · M. Banno · T. Miyata
Department of Etiology and Pathogenesis,
National Cardiovascular Center Research Institute,
Suita, Japan

suppress cell surface expression of TSC. Although WNK1 per se was inactive on the transporter activity, it was able to abolish the inhibitory effect of WNK4, suggesting that both proteins act on the same signaling pathway (Wilson et al. 2003; Yang et al. 2003). Thus, WNK4 functions as a negative regulator for the surface expression of Na-Cl cotransporter, and loss of this regulation can cause an inherited form of hypertension. WNK1 seems to act as a suppressor of WNK4, and gain-of-function of this gene can cause loss in WNK4 function leading to an inherited form of hypertension.

It is likely that individual BP level is influenced by several different genetic variants in a general population. A polymorphism in *WNK4* (base1156666G>A) has been reported to be associated with hypertension in a Caucasian population (Erlach et al. 2003) with a discrepancy in other studies (Benjafield et al. 2003; Speirs and Morris 2004). We hypothesized that the genetic polymorphisms in *TSC*, *WNK1*, and *WNK4* may involve changes in BP level. Among the different kinds of genetic variations, single nucleotide polymorphisms (SNP) receive much attention due to their easy genotyping. This study was undertaken to identify genetic variations, mainly SNPs, in all exons of *TSC*, *WNK1*, and *WNK4* and to examine the association of SNPs with hypertension in a Japanese general population.

Methods

Subjects

The subjects of the Suita study consisted of 14,200 men and women (30–79 years of age), who had been randomly selected from the municipal population registry considering group stratification by gender and 10-year age. They were all invited, by letter, to have a group checkup every 2 years at the Division of Preventive Cardiology, National Cardiovascular Center, Japan. DNA from the leukocytes was collected from participants who visited the National Cardiovascular Center between April 2002 and February 2003. The study protocol was approved by the ethical committees on human research of the National Cardiovascular Center and Suita city. Written informed consent was obtained from each subject for proceeding genetic analyses. In this study, the genotypes of 1,818 individuals including 771 subjects with hypertension (396 men and 375 women) and 1,047 controls (439 men and 608 women) were performed.

Measurements

BP was measured after at least 10 min of rest in a sitting position. Systolic and diastolic BPs (SBP and DBP) were the means of two measurements by well-trained doctors using a mercury sphygmomanometer (recorded in a 3 min pause). Hypertension was defined as SBP of ≥ 140 mmHg, DBP of ≥ 90 mmHg or current use of antihypertensive medication.

A physician or nurse questioned each patient regarding current smoking and alcohol drinking habits and personal history of cardiovascular disease, including angina pectoris, myocardial infarction, and/or stroke. Hypercholesterolemia was defined as total serum cholesterol levels ≥ 5.68 mmol/l (≥ 220 mg/dl) or current use of antihyperlipidemic medication. Diabetes was defined as fasting plasma glucose levels ≥ 7.0 mmol/l (126 mg/dl) or nonfasting glucose levels ≥ 11.1 mmol/l (200 mg/dl), HbA1C $\geq 6.5\%$, or current use of antidiabetic medication. Body mass index (BMI) was calculated as weight (in kilograms) divided by height (in meters) squared.

Blood samples from the subjects after 12 h of fasting were collected in EDTA-containing tubes. Total cholesterol and high density lipoprotein (HDL) cholesterol levels were measured with an autoanalyzer (Toshiba TBA-80) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

Direct sequencing for SNP discovery and genotyping of polymorphisms

For DNA sequencing, Japanese patients with essential hypertension at the Division of Hypertension and Nephrology, National Cardiovascular Center, Japan, were recruited. Genomic DNA was extracted using an NA-3000 nucleic acid isolation system (KURABO, Osaka, Japan). We sequenced the 48 or 96 Japanese hypertensive samples in which hypertension-susceptible SNPs would be most concentrated. In exon 22 of *TSC* and exons 7 and 17 of *WNK4*, more than 250 Japanese hypertensive samples were sequenced (Kamide et al.

Table 1 Basic characteristics of subjects in Suita, a Japanese urban population, 2002. HDL high density lipoprotein cholesterol. Values are mean $\pm$ SD or percentage. Hypertension indicates SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg or antihypertensive medication. Hyperlipidemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidemia medication. Diabetes, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl) or nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl) or antidiabetic medication

	Women (n=983)	Men (n=835)
Age (year)	63.3 $\pm$ 11.0	66.3 $\pm$ 11.1*
Systolic blood pressure (mmHg)	128.0 $\pm$ 19.6	131.9 $\pm$ 19.5*
Diastolic blood pressure (mmHg)	76.6 $\pm$ 9.8	79.7 $\pm$ 10.7*
Body mass index (kg/m ²)	22.3 $\pm$ 3.2	23.3 $\pm$ 3.0*
Total cholesterol (mmol/l)	5.57 $\pm$ 0.79*	5.10 $\pm$ 0.78
HDL cholesterol (mmol/l)	1.67 $\pm$ 0.40*	1.42 $\pm$ 0.36
Current smokers (%)	6.3	30.1**
Current drinkers (%)	29.3	67.0**
Present illness (%)		
Hypertension	38.2	47.4**
Hyperlipidemia	55.2**	27.4
Diabetes mellitus	5.2	12.6**

* $P < 0.05$ between men and women by Student's t test

** $P < 0.05$ between men and women by χ^2 test

Table 2 List of 108 polymorphisms and their allele frequencies in *TSC*, *WINK1*, and *WINK4* genes identified by direct sequencing. dbSNP ID was searched by using SNPper, a CHIP Bioinformatics Tool (Riva and Kohane 2001; <http://snpper.chip.org/bio/snpenter>, as of May 1 of 2003, that was constructed by dbSNP build 112). The apparent linkage disequilibrium (LD), defined by r^2 more than 0.5, is indicated by *a–l* in the LD column. Exon 22 of *TSC* and exons 7 and 17 of *WINK4* were sequenced using more than 250 hypertensive samples

Gene name	Allele 1/ allele 2 SNPs	LD ($r^2 > 0.5$)	Amino acid change	Region	Allele 1 homo	Hetero	Allele 2 homo	Total	Allele frequency		Flanking sequence	dbSNP ID
									Allele 1	Allele 2		
<i>TSC</i>	<i>C-1991A</i>	<i>a</i>		Promoter	38	10	0	48	0.896	0.104	CACCACCTGCTC[C]/A[CTGCAATGGCTT	
	<i>A-950G</i>	<i>b</i>		Promoter	1	19	21	41	0.256	0.744	TTTAATAGAGAG[C]/G[GGGTTTACCAT	
	<i>C-704T</i>			Promoter	46	1	0	47	0.989	0.011	CAGACGCCCGG[C]/T[GCCACACCCCTGG	
	<i>C-605T</i>	<i>a</i>		Promoter	37	10	0	47	0.894	0.106	CACTTTGAAAAT[C]/T[CCTGTCTCTGTT	
	<i>C-553T</i>			Promoter	26	1	0	27	0.981	0.019	AGCCCCAGTCA[C]/TGTACCCCTGCT	
	<i>-544delT</i>			Promoter	47	1	0	48	0.990	0.010	TCACGTACCCCTT[-]GCTTGCTCAATC	
	<i>C-213G</i>	<i>a</i>		Promoter	35	8	0	43	0.907	0.093	GGAGTGGCTGG[C]/G[TTTGGGCAGCC	
	<i>C-142T</i>	<i>b</i>		Promoter	1	20	22	43	0.256	0.744	GTGTTTGCCTC[C]/T[GGCCCTGTCCGG	
	<i>G-141C</i>	<i>b</i>		Promoter	28	15	0	43	0.826	0.174	TGTTCTGCCTCC[G]/C[GCCCTGTCCGG	
	<i>C1784T</i>			Intron 1	30	17	1	48	0.802	0.198	TGGATGCAGAG[A]/T[GGCTCCTCTAGC	
	<i>A1918G</i>		Ala122Ala	Exon 2	31	17	0	48	0.823	0.177	GGAGGCGAGG[C]/G[GGCACCAGCAGC	rs2304479
	<i>A2141T</i>			Intron 2	0	8	40	48	0.083	0.917	ACAAATAGATTAA[A]/T[GCCTGCCGGGA	rs2304480
	<i>G2971A</i>			Intron 2	47	1	0	48	0.990	0.010	TAGGGCTAGGT[G]/A[CTCGATACCTTG	
	<i>C4527A</i>		Thr180Lys	Exon 4	43	2	0	45	0.978	0.022	TGCTGCGGTCA[C]/A[GGTGACCTCCAT	
	<i>C7479T</i>		Phe341Phe	Exon 8	38	2	0	40	0.975	0.025	TGGACCTTCTT[C]/T[GGAAATGTTCTCC	
	<i>C14272T</i>	<i>c</i>		Intron 10	26	18	3	47	0.745	0.255	CTGGCTCAGCC[C]/T[CACCGTGGAGTC	
	<i>G14277A</i>			Intron 10	46	1	0	47	0.989	0.011	TCAGCCCCAC[C]/A[TTGGAGTCCCTGA	
	<i>C14363A</i>		Ala464Ala	Exon 11	45	2	0	47	0.979	0.021	CATCTTCGGGG[C]/A[ACCTCTCTCTCT	rs3816119
	<i>C14366T</i>		Thr465Thr	Exon 11	46	1	0	47	0.989	0.011	CTTCGGGGCA[C]/T[CTCTCTCTCTGCC	rs5801
	<i>G17337A</i>			Intron 13	44	1	0	45	0.989	0.011	GGGTGGGAGT[G]/A[GAGGACATGGGTG	
	<i>T18806C</i>			Intron 13	6	24	18	48	0.375	0.625	GACTGTGGCTT[T]/C[GGCCACAGGTGG	rs2304483
	<i>C18850T</i>	<i>d</i>	Ala569Val	Exon 14	46	2	0	48	0.979	0.021	ACAAAGATGG[C]/T[GGCGCTGTTTGG	
	<i>T20072C</i>		Leu623Pro	Exon 15	46	1	0	47	0.989	0.011	GCTCTACAACTT[C]/G[GGCCCTCAGTA	
	<i>G20088A</i>		Ser628Ser	Exon 15	46	1	0	47	0.989	0.011	CCTCAGTACT[C]/A[GTGGGCTCAAT	
	<i>C20201G</i>			Intron 15	46	1	0	47	0.989	0.011	GAGTTTCCAAG[C]/G[TAGACCTGTCACT	
	<i>G21421A</i>	<i>e</i>		Intron 16	20	24	3	47	0.681	0.319	ATGGGGGCCAA[G]/A[GGGATGCGGAGC	
	<i>C21500T</i>			Intron 16	42	2	0	44	0.977	0.023	CCCTTGTGTC[C]/T[TTCTCCCCCAGC	
	<i>C21566G</i>			Intron 16	43	1	0	44	0.989	0.011	CACCTTCTCCC[C]/G[ACTCCTTGTGTT	
	<i>A21586G</i>	<i>c</i>	Ala714Ala	Intron 16	43	1	0	44	0.989	0.011	GTGTTTCCCCTT[A]/G[TTCTGGGCAAAAG	rs3764264
	<i>C21822T</i>			Exon 17	21	21	3	45	0.700	0.300	GGATGTCATTG[C]/T[AGAGACCTCCGC	
	<i>C22682T</i>			Intron 17	46	1	0	47	0.989	0.011	TCACCCCTATCC[C]/T[CTGGCAGGCCGC	
	<i>C25013T</i>	<i>c</i>		Intron 18	23	22	3	48	0.708	0.292	CTGGGGAGAA[G]/T[TTGGACCTCACCT	
	<i>G27029A</i>	<i>c</i>		Intron 20	18	25	4	47	0.649	0.351	TTTTCTGTGAC[C]/A[GTGGTGCCTGAG	
	<i>C27646T</i>	<i>d</i>		Intron 20	6	26	15	47	0.404	0.596	AAGGGCGTTGG[C]/T[GGGCCCTGGGC	rs2278490
	<i>T27681C^a</i>	<i>d</i>		Intron 20	5	23	18	47	0.351	0.628	TGGATGCGCGG[C]/C[GTGGCTCTGCT	rs2278489
	<i>A27681C^a</i>			Intron 20	0	1	0	1	0.011		TGGATGCGCGG[C]/C[GTGGCTCTGCT	
	<i>T27681A^a</i>					0	0				TCATCCCTATCT[A]/CCTTGTGGCCGCAA	
	<i>T29320A</i>		Leu849His	Exon 22	367	5	0	372	0.993	0.007	TGTGTCGTAGG[C]/T[GGCCAGATTAACT	rs5804
	<i>C29372T</i>	<i>c</i>	Gly866Gly	Exon 22	23	22	3	48	0.708	0.292	TCTCAAGAAAA[G]/A[TAATAACAATAAA	
	<i>G34262A</i>	<i>f</i>		Intron 22	44	1	3	48	0.927	0.073	ACCAGAACCTT[C]/A[GGCTGAGCAGTA	
	<i>G34372A</i>	<i>g</i>	Arg904Gln	Exon 23	45	3	0	48	0.969	0.031	CACAGGGCAA[G]/T[GGTGCAGCCCC	
	<i>C34588T</i>	<i>f</i>		Intron 23	41	3	4	48	0.885	0.115	CCTCAACCCACTT[C]/TCTCTGCCCCAG	
	<i>T37125C</i>			Intron 23	46	1	0	47	0.989	0.011	GGCCACTGTCAA[C]/T[AGATGCGGCGG	
	<i>C37210T</i>		Asn931Asn	Exon 24	46	1	0	47	0.989	0.011		

Table 2 (Continued)

Gene name	Allele 1/ allele 2 SNPs	LD (r ² > 0.5)	Amino acid change	Region	Allele 1 homo	Hetero	Allele 2 homo	Total	Allele frequency Allele 1 Allele 2	Flanking sequence	dbSNP ID	
WNK1	A37311G	e		Intron 24	23	21	3	47	0.713	0.287	ACGGACACATC[A/G]CTGGGTCAGGGA	rs2289117
	G39097A			Intron 24	29	1	0	30	0.983	0.017	GAGCCATAGAC[G/A]TGGTGAAGGATT	
	C39119T			Intron 24	29	1	0	30	0.983	0.017	ATTGAGTGACCT[C/T]GATGATATGGGA	
	C39142T			Intron 24	40	7	0	47	0.926	0.074	GAAGTGACCACT[C/T]GGCTTCTCCCG	rs3816118
	G39143A	g		Intron 24	44	3	0	47	0.968	0.032	AAAGTGACCACT[G/A]GCTTCTCCCGC	rs2289116
	C39203T		Ser96Phe	Exon 25	46	1	0	47	0.989	0.011	TGCTGGAATTACT[C/T]CCGAGACGCTGC	
	C39240T	g		Intron 25	43	4	0	47	0.957	0.043	GTAAGTAGTGCC[C/T]GGCTGGTGGGAG	rs2289115
	C39375T	e		Intron 25	23	20	4	47	0.702	0.298	ACATAGCCCTGG[C/T]GATTTCTAGCAT	rs2289114
	C48128T		Ile1008Ile	Exon 26	38	9	0	47	0.904	0.096	AGTCATCCTGAT[C/T]CGAGGAAACACAG	rs2289113
	A48195G		3'UTR	Exon 26	46	1	0	47	0.989	0.011	ACATCCCTGTCCA[G/C]AGCTCTGAGTG	
	G421A		Ala141Thr	Exon 1	89	5	0	94	0.973	0.027	CCTCAGCCGCT[G/A]CCGCCCTGGGG	
	C446T		Ala149Val	Exon 1	90	4	0	94	0.979	0.021	AACAGGCCGTGC[C/T]GGGCCCTGCCCC	
	C511T		Leu171Phe	Exon 1	93	1	0	94	0.995	0.005	TCCAGCCTAGC[C/T]TGTGGGGAGCA	rs3858703
	G786A	a,b,c		Intron 1	0	15	80	95	0.079	0.921	ACTTATTGAC[G/A]GTCCTTTGGAATC	
	A59884G			Intron 1	88	1	0	89	0.994	0.006	TCTGAGTTACAC[A/G]TTAACAGTAAAG	
	C73737G	a,b,c		Intron 3	0	16	79	95	0.084	0.916	GACTGGCTTCT[C/G]ACATTCTCTTTA	rs2158502
A76571G	a,b	Ala429Ala	Exon 4	0	16	78	94	0.085	0.915	CCAAAATGCTG[C/A/G]CAGATCTACCGT		
C105668A	d		Intron 5	91	4	0	95	0.979	0.021	TTCTTTTCCCT[C/A]TGTGTTGGAAGT		
T105758C	d	Asp493Asp	Exon 6	91	4	0	95	0.979	0.021	AGCAGAAAGAA[G/T]CJGATGGAGAAAA	rs2286006	
G105987A			Intron 6	93	1	0	94	0.995	0.005	TGATGAAGTGCC[G/A]TGTGTGGCATAT		
A107419G			Intron 6	75	13	0	88	0.926	0.074	TTTCAATATACT[A/G]CTGCTTAAATTA		
C108560T	a,c	Thr665Ile	Exon 8	85	10	0	95	0.947	0.053	CCTCTGTCTTCA[C/T]AGAATCTCGAGT	rs2286007	
G124751A	e,f,g	Gln776Gln	Exon 10	4	26	56	86	0.198	0.802	GCCAGTGAGTCA[G/A]CCTCAAGCTCCA	rs1012729	
T125972A			Intron 10	92	1	0	93	0.995	0.005	TTTTTTTTTTT[A/A]AGCCTGTCTGT		
G126163A	h	Gln843Gln	Exon 11	75	20	1	96	0.885	0.115	CCCTGCTCTCA[G/A]ATTCCTCATATCA		
A128177C	i	Thr1056Pro	Exon 13	3	19	71	93	0.134	0.866	GCAGTAGCACAG[A/G]CCCCAAGCTACCC	rs956868	
C128274T	e,f,g		Intron 13	60	28	5	93	0.796	0.204	GACGGTATGAA[A/C/T]GCCAAACTGTCA		
C129494T	h		Intron 16	74	20	1	95	0.884	0.116	ACAATTATGTA[C/T]GTCTGCAATTGG		
A129852G		Ile1172Met	Exon 16	88	4	0	92	0.978	0.022	TATCTAGCAAT[A/G]AGAGAGAGATCG		
C130104T			Intron 16	90	2	0	92	0.989	0.011	GACACCATGAC[C/T]GACAAACAAACTT		
T130917G	e,f,g,j		Intron 18	44	39	12	95	0.668	0.332	GATATTGATGAT[T/G]GTGTTTATTCT		
C131195T	f,g,j,k	Asn1320Asn	Exon 19	20	47	28	95	0.458	0.542	AGAAGGACCCA[A/C/T]ACAGCACCTCCA		
C131279T	i	Thr1348Thr	Exon 19	72	19	3	94	0.867	0.133	TGGAGTCCCAA[C/T]ACAGCAGCAGCC		
C132236T		Ser1667Ser	Exon 19	87	2	0	89	0.989	0.011	CAGTGAAACACAG[C/T]TCACTCTGGAGCT		
C132444G		Pro1737Ala	Exon 19	88	1	0	89	0.994	0.006	CAAGTTTCTACC[C/G]CAGTCACGACTA		
I32576delT	i		Intron 19	68	17	3	88	0.869	0.131	ATCAGTTTTTTT[-]CTCCCTAATGAG		
A132655G	g,j,k		Intron 19	20	36	15	71	0.535	0.465	CTTATGATTTT[A/G]TTAAATTGACAG		
C133634T	h		Intron 19	72	19	0	91	0.896	0.104	TTTAGCGTCTCA[C/T]GGACTTGATTT		
G135642T	e,f,g,j,k	Met1808Ile	Exon 21	42	42	9	93	0.677	0.323	TAGTCCAGAGAT[G/T]ATCACAGTGACT		
T135771G			Intron 21	92	1	0	93	0.995	0.005	TTTAAATGTAT[T/G]CAGAGATTCTCTGC		
G136943A		Gln1832Gln	Exon 22	93	1	0	94	0.995	0.005	AGCAGGAACACA[G/A]CCTCAGAAAGGT		
A141069T		Gly1858Gly	Exon 23	86	3	0	89	0.983	0.017	TTTAAAGATGGG[A/T]CGATTTCAGGTA	rs2301880	
C141114T			Intron 23	58	27	4	89	0.803	0.197	CTTGATTCCTT[C/T]TTTGGAGGAGTT		
T142439C	e,f,g		Intron 23	70	19	1	90	0.883	0.117	TGATCTTTTTTTT[C/C]CTTTTTTAAAT		
C142763T	h	Arg1945Cys	Exon 24	87	6	0	93	0.968	0.032	ACCAAGTTTGA[C/T]GTTTTACGGTGA		
C163T		Arg55Cys	Exon 1	95	1	0	96	0.995	0.005	GAGCCCCGGCC[C/T]GCTCTTCTCGTC		
G288A		Arg96Arg	Exon 1	95	1	0	96	0.995	0.005	TGGCCCCGGCAG[G/A]AGCCCCACCGCCT		
C383T		Pro128Leu	Exon 1	95	1	0	96	0.995	0.005	GTCCCCGAGCTCC[C/T]GGACTCTGCAGT		
WNK4												

T2074C	Ser211Ser	Exon 2	93	1	0	94	0.995	0.005	TCGGAACACTGCTT/CJAGAGCTGAGCGG
C2285T		Intron 2	87	7	0	94	0.963	0.037	GATGTGTGCCCA/C/TJTGCTTCCTGAAC
A4732G	Ile474Val	Exon 6	94	1	0	95	0.995	0.005	GACAAACAGGCC/A/GJTCGAGTTCTGT
A6744G	Met546Val	Exon 7	277	1	0	278	0.998	0.002	GCAACTGTGCCA/A/GJTGCCCCCGGTC
C6749T	Ala567Ala	Exon 7	87	5	1	93	0.962	0.038	TGTGCCCATGGC/C/TJCCCCGTCCCCC
G7144T	Ala601Ser	Exon 8	89	6	1	96	0.958	0.042	GCCTCAGACCTT/G/TJCCCTTCAGCCCC
A7235		Intron 8	83	12	1	96	0.927	0.073	TGGGGGCTCC/C/A/DELJGCCATTCCAAGC
G8119A		Intron 11	95	1	0	96	0.995	0.005	GAGGGGAGAGA/G/AJATGAGACAGAG
G12806C		Intron 12	89	6	1	96	0.958	0.042	CGCGCCACGCTT/CJATGTTTAAAGAT
T12948C	Ile740Thr	Exon 12	95	1	0	96	0.995	0.005	GGATTGGGAGA/T/CJATCCAGCAGT
G14139C	Gly808Ala	Exon 14	90	1	0	91	0.995	0.005	CATCTTCTCTG/C/AACTCCCTTTGTC
G14440A	Pro908Pro	Exon 14	89	6	1	96	0.958	0.042	TTTCTTCTCTG/C/ATGCCCTCCACT
C14597T	Pro961Ser	Exon 14	88	6	1	95	0.958	0.042	CCTAGTCCCTC/C/TJCTAGCCTGCCCC
C14717T		Intron 14	75	19	0	94	0.899	0.101	AGGAGACTCCA/C/TJCTGCACCTCTC
C15503A	Pro1173Thr	Exon 17	278	1	0	279	0.998	0.002	AAGCAGCCCCA/C/AJCGGATTTGTG
T15677C		Intron 17	275	2	0	277	0.996	0.004	CTGTCGACTGTT/CJTTCTCCAGGCC
C15703T		Intron 17	277	1	0	278	0.998	0.002	GGGGTCTGCCC/C/TJGGGGGAATAGAC
C15738A		Intron 17	272	4	0	276	0.993	0.007	CACCTCCCTTT/C/AJCTCACTTAGTGC

^aTriallelic polymorphism

2004). The method of direct sequencing was described previously (Okuda et al. 2002). The polymorphisms were identified by use of Sequencer software (Gene Codes Corporation, Ann Arbor, MI, USA) and confirmed by visual inspection (Takiuchi et al. 2004). SNPs having a minor allele frequency of greater than 5% were defined as candidates for genotyping using the TaqMan-PCR system (Tanaka et al. 2003). Some SNPs were not suitable for genotyping due to the presence of another SNP in the adjacent region. The representative SNPs were genotyped when they were in linkage disequilibrium (r^2 over 0.5). Since a missense mutation may directly be susceptible to hypertension, five missense SNPs with minor allele frequencies below 5%, including C4527A (Thr180Lys, *TSC*), T29320A (Leu849His, *TSC*), G34372A (Arg904Gln, *TSC*), C142763T (Arg1945Cys, *WNK1*), and C15503A (Pro1173Thr, *WNK4*), were also genotyped.

Statistical analysis

A total of 1,818 subjects who had complete genotype data were recruited for the study. Analysis of variance was used to compare mean values between groups, and if overall significance was demonstrated, the intergroup difference was assessed by means of a general linear model. Frequencies were compared by χ^2 analysis.

Association studies of genotypes with BP were performed through logistic regression analysis considering potential confounding variables in risk factors, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive drug use by gender. For multivariate risk factors, adjusted odds ratios were given with 95% confidence intervals. The associations of genotypes with hypertension were expressed in terms of odds ratios adjusted for possible confounding effects, including age, BMI, present illness (hyperlipidemia and diabetes mellitus), and lifestyle (smoking and drinking) by gender. All analyses were performed with SAS statistical software (release 8.2, SAS Institute, Inc., Cary, NC, USA). Linkage disequilibrium was calculated by using the SNPalyze version 2.1 (DYNACOM Co., Ltd, Mobara, Japan).

Results

Basic characteristic of subjects

The characteristics of all 1,818 participants (835 men and 983 women) are shown in Table 1. Age, SBP, DBP, BMI, percentage of current smokers, percentage of current drinkers, and prevalence of individuals with hypertension and diabetes mellitus were significantly higher in men than in women. Total cholesterol, HDL cholesterol, and percentage of individuals with hyperlipidemia were significantly higher in women than in men.

Table 3 Primers and TaqMan probes for genotyping of SNPs in *TSC*, *WNK1*, and *WNK4*

Gene	SNP	Primer	Probe
<i>TSC</i>	<i>C-1991A</i>	CCCTGACAGCTCAAATTTCCAC	Fam-CTGCCTCCCTGCAA-MGB
		CTTGTTACCAGAGGTGCCTAAGC	Vic-CTGCCTCACTGCAA-MGB
	<i>C-605T</i>	GCAGAAATGAAATCCACAAGCA	Fam-TTTGAAAATCCCTGTCTG-MGB
		CATGCACCGATCATTAGATTGG	Vic-CTTTGAAAATCCCTGTCTG-MGB
	<i>C-213G</i>	GGCAGAACACCATTTGATTGTG	Fam-CTGGCCCAAAGCCAGCCACTC-TAMRA
		GAAGAGCCACTCCAGGACTCA	Vic-CTGGCCCAAACCCAGCCACTC-TAMRA
	<i>C1784T</i>	CGCAGTGGTGCAGGTCAGT	Fam-CAGAGACGCCGTCC-MGB
		AGGTGTCTGCCTTCCTGCTG	Vic-TGCAGAGATGCCGTCC-MGB
	<i>A1918G</i>	CTCACCATCACCCCCTTGAC	Fam-CTGGTGCCTGCCTCGCCC-TAMRA
		CAGCAGGAAGGCAGACACCT	Vic-TGGTGCCTGCCTCGCC-TAMRA
	<i>A2141T</i>	GCTTCAGTTTCCCCATCTGTACA	Fam-AATAGATTAAAGCCTGCCGG-MGB
		GGTGGCTTTTATAGGAAACACA	Vic-AATAGATTAAATGCCTGCCGG-MGB
	<i>C4527A</i>	GATGAACGTAGGTCGCTATGGT	Fam-TGTCGGTCAAGGTG-MGB
		GATGGCTGAGATGGAGAGGC	Vic-TGTCGGTCAAGGTG-MGB
	<i>T18806C</i>	AGCAGCTCTGGCCTAGAAAGAG	Fam-TGGTGCCCTTGGCCAGG-TAMRA
		ACGGAGATGATAGCCCCAAG	Vic-CTGGTGCCCTTGGCCAGG-TAMRA
	<i>T29320A</i>	TCACATAGTGCTCTGTCTGAGTG	Fam-TCCCTATCTCCTTGGC-MGB
		GATCTTGCATTTGCTCCACCTC	Vic-CCTATCACCTTGGCC-MGB
	<i>C29372T</i>	GCAAGAGGAGGTGGAGCAAAT	Fam-TTCGTAGGCGGCCAG-MGB
		CCCTCCACACTTACGCCTTTC	Vic-TCGTAGGTGGCCAGAT-MGB
	<i>G34372A</i>	GGGATTCCATGAAGTCCACATC	Fam-AACCCTCGGGCTGA-MGB
		CTGGAAGCCCCAAAACAGAAC	Vic-AGAACCCTCAGGCTG-MGB
<i>WNK1</i>	<i>C39375T</i>	GAAGCAGAAGGGCCAAAGTTC	Fam-ATAGCCCTGGCGATT-MGB
		GATGCCTGGGACACGTGAG	Vic-TAGCCCTGGTGATTG-MGB
	<i>G786A</i>	GAAGTGCAGGTAAAGCCCCAC	Fam-TTTGACGGTCTTTG-MGB
		GAACTCGATCAACTGGCTTCG	Vic-TTTATTGACAGTCTTTG-MGB
	<i>C108560T</i>	CTGATGGGACGGTTGACAGTG	Fam-TCTTCACAGAATCTCGA-MGB
		CCTGTTTCATGTTGGGAACCATA	Vic-TCTTCATAGAATCTCG-MGB
	<i>A128177C</i>	GTTGCTCCTGCAGAGCCAGT	Fam-AGTAGCACAGACCCAA-MGB
		TCTACAGAGGAAGCCAAAGTGGT	Vic-AGTAGCACAGCCCA-MGB
	<i>C133634T</i>	TTGATTTGCTCTTCAGTACGCAG	Fam-AGCGTCTCAGGACT-MGB
		GCACCTACAGACAACAAAGGGAA	Vic-AGCGTCTCATGGACT-MGB
<i>WNK4</i>	<i>G135642T</i>	AAAACCTACACCAACCGCAGAAG	Fam-CTGTGATCATCTCTG-MGB
		ATTCAGTCCCAGCAACCTCTAGA	Vic-ACTGTGATAATCTCTG-MGB
	<i>C141114T</i>	TGGGACGATTTTCAGGTAAGACAG	Fam-ATTCCTTCTTTGGAGGA-MGB
		TTGTGTCCCAAATAGGTAGGCA	Vic-ATTCCTTCTTTGGAGGAG-MGB
	<i>C142763T</i>	ACGACCCACTTTGTTTGCTGTA	Fam-CTGAAAACGTCCAACCT-MGB
		GTCAGACACTGGGCAGCCTAC	Vic-CCTGAAAACATCCAACCT-MGB
	<i>C14597T</i>	CTGGCTGTGATGACTGTGGC	Fam-TCCCCCTCCTAGCCT-MGB
		TGAAGGGCTTTCTTGCC	Vic-TCCCCCTCTAGCCTG-MGB
	<i>C14717T</i>	CACAGCTGAGGTGGAGAGTGAG	Fam-CTCCACTCTGCACTC-MGB
		GGAGGTGGTGAGGCCTAGAAA	Vic-ACTCCATTCTGCACTC-MGB

Polymorphisms of *TSC*, *WNK1*, and *WNK4*

We sequenced 96 alleles from 48 patients with hypertension in *TSC* and 192 alleles from 96 patients with hypertension in *WNK1* and *WNK4*, and identified 52, 35, and 21 polymorphisms, respectively (Table 2). There were six, nine, and nine missense mutations in *TSC*, *WNK1* and *WNK4*, respectively. Among them, missense mutations with minor allele frequencies above 5% were 0, 3, and 0, respectively, indicating that most of the missense mutations were rare. We selected SNPs with minor allele frequencies above 5% for genotyping. Five missense SNPs with the minor allele frequency below 5% were also included. We selected representative SNPs for genotyping when some of the SNPs were in linkage disequilibrium. Finally, 12, 7, and 2 SNPs, in a total of 21 SNPs, were selected for genotyping in population-based samples. The primers and probes of the TaqMan-PCR method are summarized in Table 3.

Susceptible SNPs related to hypertension

The results of the case-control study are shown in Table 4. Among 21 SNPs, the C14717T SNP of *WNK4* was significantly associated with hypertension in men ($\chi^2=7.53$, $p=0.023$). SBP in men with the CT+TT genotypes was 3.1 mmHg higher than those with the CC genotype ($p=0.042$) after adjustment for age, BMI, hyperlipidemia, diabetes mellitus, antihypertensive drug use, smoking, and drinking (Table 5). Multivariate logistic regression analysis with adjustment for age, BMI, hyperlipidemia, diabetes mellitus, smoking, and drinking revealed that the odds ratio for the presence of hypertension for the CT+TT genotypes in C14717T in comparison to the CC genotype in men was 1.62 (95% confidence interval, 1.12–2.33, $p=0.010$) (Table 6). When the controls were defined as SBP ≤ 120 mmHg, DBP ≤ 80 mmHg, or nonmedication, and the hypertensives were defined as SBP ≥ 160 mmHg, DBP ≥ 100 mmHg, or current use of antihypertensive

Table 4 Genotype distributions of 21 SNPs of *TSC*, *WNK1*, and *WNK4* in normotensives and hypertensives. *n.d.* not determined

Gene	SNP	Genotypes	Women (<i>n</i> =983)				Men (<i>n</i> =835)			
			Normotensive (<i>n</i> =608)	Hypertensive (<i>n</i> =375)	χ^2	<i>p</i>	Normotensive (<i>n</i> =439)	Hypertensive (<i>n</i> =396)	χ^2	<i>p</i>
<i>TSC</i>	C-1991A	(CC/CA/AA)	539/67/2	337/37/1	0.359	0.836	392/45/2	359/36/1	0.571	0.752
<i>TSC</i>	C-605T	(CC/CT/TT)	539/67/2	337/37/1	0.359	0.836	392/45/2	359/36/1	0.571	0.752
<i>TSC</i>	C-213G	(CC/CG/GG)	539/67/2	337/37/1	0.359	0.836	392/45/2	359/36/1	0.571	0.752
<i>TSC</i>	C1784T	(CC/CT/TT)	435/161/12	289/81/5	3.754	0.153	320/112/7	293/94/9	0.800	0.670
<i>TSC</i>	A1918G	(AA/AG/GG)	407/175/26	240/118/17	0.900	0.638	283/133/23	253/131/12	2.945	0.229
<i>TSC</i>	A2141T	(AA/AT/TT)	6/114/488	4/67/304	0.131	0.936	2/85/352	3/71/322	0.579	0.749
<i>TSC</i>	C4527A	(CC/CA/AA)	591/17/0	362/13/0	<i>n.d.</i>	<i>n.d.</i>	427/12/0	382/14/0	<i>n.d.</i>	<i>n.d.</i>
<i>TSC</i>	T18806C	(TT/TC/CC)	115/285/208	63/181/131	0.703	0.704	57/210/172	50/182/164	0.435	0.804
<i>TSC</i>	T29320A	(TT/TA/AA)	592/16/0	360/15/0	<i>n.d.</i>	<i>n.d.</i>	428/11/0	391/5/0	<i>n.d.</i>	<i>n.d.</i>
<i>TSC</i>	C29372T	(CC/CT/TT)	325/242/41	199/143/33	1.475	0.478	36/186/36	213/155/28	1.645	0.439
<i>TSC</i>	G34372A	(GG/GA/AA)	548/59/1	334/40/1	0.362	0.835	387/50/2	347/49/0	<i>n.d.</i>	<i>n.d.</i>
<i>TSC</i>	C39375T	(CC/CT/TT)	342/222/44	207/146/22	1.057	0.589	231/174/34	189/161/46	4.302	0.116
<i>WNK1</i>	G786A	(GG/GA/AA)	9/133/466	4/93/278	1.356	0.508	7/82/350	7/82/307	0.602	0.740
<i>WNK1</i>	C108560T	(CC/CT/TT)	527/76/5	310/62/3	3.127	0.209	377/60/2	342/52/2	0.061	0.970
<i>WNK1</i>	A128177C	(AA/AC/CC)	9/135/464	4/80/291	0.430	0.807	9/102/328	8/86/302	0.280	0.869
<i>WNK1</i>	C133634T	(CC/CT/TT)	453/143/12	267/101/7	1.449	0.485	335/94/10	296/93/7	0.733	0.693
<i>WNK1</i>	G135642T	(GG/GT/TT)	244/290/74	139/182/54	1.478	0.478	196/187/56	164/182/50	1.040	0.595
<i>WNK1</i>	C141114T	(CC/CT/TT)	361/218/29	219/134/22	0.576	0.750	278/135/26	241/134/21	0.962	0.618
<i>WNK1</i>	C142763T	(CC/CT/TT)	592/16/0	362/13/0	<i>n.d.</i>	<i>n.d.</i>	421/17/1	389/7/0	<i>n.d.</i>	<i>n.d.</i>
<i>WNK4</i>	C14597T	(CC/CT/TT)	581/27/0	353/22/0	<i>n.d.</i>	<i>n.d.</i>	410/29/0	375/21/0	<i>n.d.</i>	<i>n.d.</i>
<i>WNK4</i>	C14717T	(CC/CT/TT)	466/131/11	303/67/5	2.394	0.302	367/68/4	303/84/9	7.526	0.023

medication, the C14717T polymorphism was significantly associated with hypertension in men (CC vs CT+TT, odds ratio=1.91, 95% confidence interval: 1.02–3.58, *p*=0.045) after adjustment for the confounding factors described above.

Discussion

Three genes, *TSC*, *WNK1*, and *WNK4*, are potentially strong candidates for essential hypertension (Choate et al. 2003; Wilson et al. 2003). To understand whether these genes influence BP, we sequenced these genes and identified a total of 108 SNPs. To evaluate the association of the SNPs with hypertension, we genotyped 21 representative SNPs in a large members of 1,818 individuals and identified that the C14717T polymorphism in intron 14 in *WNK4* was associated with hypertension in men. The TT genotype of this SNP increased SBP by 3.1 mmHg when compared with the CC+CT genotype (Table 5). The association of this SNP with hypertension

was observed after multiple adjustments for confounding factors including age, BMI, present illness (hyperlipidemia and diabetes mellitus), lifestyle (smoking and drinking), and antihypertensive medication (Table 6). Therefore, we consider that the C14717T polymorphism in intron 14 in *WNK4* was associated with hypertension in our general population.

WNK4 is located on chromosome 17q21.2. Several lines of evidence indicate a region on human chromosome 17q as a gene that influences BP (Baima et al. 1999; Jacob et al. 1991; Levy et al. 2000). A quantitative trait locus of hypertension on the rat chromosome 10, equivalent to human chromosome 17, was identified in spontaneous hypertensive rats (Jacob et al. 1991). This region was reportedly linked with hypertension using hypertensive sib pairs from the United Kingdom and France (Julier et al. 1997) and was confirmed in a study of white American hypertensive sib pairs (Baima et al. 1999). Evidence obtained from the Framingham Heart Study indicated that this region is associated with BP with LOD score of 4.7 (Levy et al. 2000). Thus, these

Table 5 Blood pressure levels on genotype of *WNK4* C14717T polymorphism. Values are means $\pm$ SDs; all adjusted for age, body mass index (BMI), antihypertensive drug use, present illness

	CC	CT	TT	<i>p</i>	CC+CT	TT	<i>p</i>	CC	CT+TT	<i>p</i>
Men (<i>n</i>)	670	152	13		822	13		670	165	
DBP	79.4 $\pm$ 0.4	81.0 $\pm$ 0.8	81.6 $\pm$ 2.7	0.052	79.7 $\pm$ 0.3	81.6 $\pm$ 2.7	0.481	79.4 $\pm$ 0.4	81.1 $\pm$ 0.8	0.051
SBP	131.3 $\pm$ 0.7	133.8 $\pm$ 1.4	140.8 $\pm$ 4.8	0.020	131.8 $\pm$ 0.6	140.8 $\pm$ 4.8	0.062	131.3 $\pm$ 0.7	134.4 $\pm$ 1.4	0.042
Women (<i>n</i>)	769	198	16		967	16		769	214	
DBP	76.6 $\pm$ 0.3	76.6 $\pm$ 0.7	76.1 $\pm$ 2.3	0.937	76.6 $\pm$ 0.3	76.1 $\pm$ 2.3	0.817	76.6 $\pm$ 0.3	76.6 $\pm$ 0.6	0.986
SBP	128.1 $\pm$ 0.6	128.1 $\pm$ 1.2	124.3 $\pm$ 4.2	0.653	128.1 $\pm$ 0.5	124.3 $\pm$ 4.2	0.358	128.1 $\pm$ 0.6	127.8 $\pm$ 1.1	0.827

(hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking); diastolic blood pressure (DBP) and systolic blood pressure (SBP) are expressed as mmHg

Table 6 Odds ratio for the presence of hypertension for *WNK4* C14717T genotype in a Japanese general population. OR odds ratio, CI confidence interval

Sex	Genotype	OR ^a (95% CI)	<i>p</i>	Genotype	OR ^a (95% CI)	<i>p</i>
Men	CC	1 (reference)	0.010	CC+CT	1 (reference)	0.079
	CT+TT	1.62 (1.12–2.33)		TT	3.00(0.88–10.19)	
Women	CC	1 (reference)	0.209	CC+CT	1 (reference)	0.621
	CT+TT	0.80(0.56–1.13)		TT	0.74 (0.22–2.45)	

^aConditional logistic analysis, adjusted for age, BMI, hyperlipidemia, diabetes mellitus, smoking, and drinking

studies suggest that this region may contain a gene susceptible for BP elevation.

The C14717T polymorphism in *WNK4* associated with hypertension was found in the intron. Therefore, it is not likely that it directly affects the function of *WNK4*, leading to hypertension. The C14717T polymorphism may be in linkage disequilibrium with another genetic variation in the region that was not examined by sequencing. The functional SNP may be present in the 5'-upstream region beyond our sequencing region or in the intron, creating a new splicing site. Further analysis is needed to clarify the function of this SNP. In conclusion, our study has shown the possible involvement of *WNK4* in essential hypertension in the Japanese general population.

Acknowledgements We would like to express our highest gratitude to Mr. Yoshio Sakaguchi, the mayor of Suita city, and Dr. Soichiro Kitamura, President of the National Cardiovascular Center, for his support of the millennium genome project. We would like to express our gratitude to Drs. Otsaburo Hishikawa, Katsuyuki Kawanishi, Tadashi Fujikawa, Akira Okayama, and Toshifumi Mannami for their continuous support of our population survey in Suita city. We also thank the members of the Satsuki-Junyukai. We thank Drs. T. Horio, Y. Miwa, M. Yoshii, Y. Miyamoto, H. Makino, K. Doi, K. Ono, and K. Shioji for obtaining informed consent for collecting blood samples. We also thank all the staff in the Division of Preventive Cardiology for supporting medical examination, and Y. Tokunaga and C. Imai for their technical assistance. This study was supported by the Program for Promotion of Fundamental Studies in Health Science of the Pharmaceuticals and Medical Devices Agency (PMDA) of Japan and a grant-in-aid (H14-027) from the Japanese Ministry of Health, Labor, and Welfare.

References

- Baima J, Nicolaou M, Schwartz F, DeStefano AL, Manolis A, Gavras I, Laffer C, Eliovich F, Farrer L, Baldwin CT, Gavras H (1999) Evidence for linkage between essential hypertension and a putative locus on human chromosome 17. *Hypertension* 34:4–7
- Benjafeld AV, Katyk K, Morris BJ (2003) Association of EDNRA, but not *WNK4* or *FKBP1B*, polymorphisms with essential hypertension. *Clin Genet* 64:433–438
- Bettinelli A, Bianchetti MG, Girardin E, Caringella A, Cecconi M, Appiani AC, Pavanello L, Gastaldi R, Isimbaldi C, Lama G et al. (1992) Use of calcium excretion values to distinguish two forms of primary renal tubular hypokalemic alkalosis: Bartter and Gitelman syndromes. *J Pediatr* 120:38–43
- Choate KA, Kahle KT, Wilson FH, Nelson-Williams C, Lifton RP (2003) *WNK1*, a kinase mutated in inherited hypertension with hyperkalemia, localizes to diverse Cl-transporting epithelia. *Proc Natl Acad Sci USA* 100:663–668
- Erlich PM, Cui J, Chazaro I, Farrer LA, Baldwin CT, Gavras H, DeStefano AL (2003) Genetic variants of *WNK4* in whites and African Americans with hypertension. *Hypertension* 41:1191–1195
- Inamoto N, Katsuya T, Kokubo Y, Mannami T, Asai T, Baba S, Ogata J, Tomoike H, Ogiwara T (2003) Association of methylenetetrahydrofolate reductase gene polymorphism with carotid atherosclerosis depending on smoking status in a Japanese general population. *Stroke* 34:1628–1633
- Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, Dzau VJ, Lander ES (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. *Cell* 67:213–224
- Julier C, Delepine M, Keavney B, Terwilliger J, Davis S, Weeks DE, Bui T, Jeunemaitre X, Velho G, Froguel P, Ratcliffe P, Corvol P, Soubrier F, Lathrop GM (1997) Genetic susceptibility for human familial essential hypertension in a region of homology with blood pressure linkage on rat chromosome 10. *Hum Mol Genet* 6:2077–2085
- Kamide K, Takiuchi S, Tanaka C, Miwa Y, Yoshii M, Horio T, Mannami T, Kokubo Y, Tomoike H, Kawano Y, Miyata T (2004) Three novel missense mutations of *WNK4*, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. *Am J Hypertens* 17:446–449
- Levy D, DeStefano AL, Larson MG, O'Donnell CJ, Lifton RP, Gavras H, Cupples LA, Myers RH (2000) Evidence for a gene influencing blood pressure on chromosome 17. Genome scan linkage results for longitudinal blood pressure phenotypes in subjects from the Framingham heart study. *Hypertension* 36:477–483
- Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN (2003) Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 33:177–182
- Mastroianni N, Bettinelli A, Bianchetti M, Colussi G, De Fusco M, Sereni F, Ballabio A, Casari G. (1996) Novel molecular variants of the Na–Cl cotransporter gene are responsible for Gitelman syndrome. *Am J Hum Genet* 59:1019–1026
- Okuda T, Fujioka Y, Kamide K, Kawano Y, Goto Y, Yoshimasa Y, Tomoike H, Iwai N, Hanai S, Miyata T (2002) Verification of 525 coding SNPs in 179 hypertension candidate genes in the Japanese population: identification of 159 SNPs in 93 genes. *J Hum Genet* 47:387–394
- Simon DB, Nelson-Williams C, Bia MJ, Ellison D, Karet FE, Molina AM, Vaara I, Iwata F, Cushner HM, Koolen M, Gainza FJ, Gitelman HJ, Lifton RP (1996) Gitelman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na–Cl cotransporter. *Nat Genet* 12:24–30
- Speirs HJ, Morris BJ (2004) *WNK4* intron 10 polymorphism is not associated with hypertension. *Hypertension* 43:766–768
- Takeuchi K, Kure S, Kato T, Taniyama Y, Takahashi N, Ikeda Y, Abe T, Narisawa K, Muramatsu Y, Abe K (1996) Association of a mutation in thiazide-sensitive Na–Cl cotransporter with familial Gitelman's syndrome. *J Clin Endocrinol Metab* 81:4496–4499
- Takiuchi S, Mannami T, Miyata T, Kamide K, Tanaka C, Kokubo Y, Koyama Y, Inamoto N, Katsuya T, Iwai N, Kawano Y, Ogiwara T, Tomoike H (2004) Identification of

- 21 single nucleotide polymorphisms in human hepatocyte growth factor gene and association with blood pressure and carotid atherosclerosis in Japanese population. *Atherosclerosis* 173:301–307
- Tanaka C, Kamide K, Takiuchi S, Miwa Y, Yoshii M, Kawano Y, Miyata T (2003) An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 26:301–306
- Wilson FH, Disse-Nicodeme S, Choate KA, Ishikawa K, Nelson-Williams C, Desitter I, Gunel M, Milford DV, Lipkin GW, Achard JM, Feely MP, Dussol B, Berland Y, Unwin RJ, Mayan H, Simon DB, Farfel Z, Jeunemaitre X, Lifton RP (2001) Human hypertension caused by mutations in WNK kinases. *Science* 293:1107–1112
- Wilson FH, Kahle KT, Sabath E, Lalioti MD, Rapson AK, Hoover RS, Hebert SC, Gamba G, Lifton RP (2003) Molecular pathogenesis of inherited hypertension with hyperkalemia: the Na–Cl cotransporter is inhibited by wild-type but not mutant WNK4. *Proc Natl Acad Sci USA* 100:680–684
- Yang CL, Angell J, Mitchell R, Ellison DH (2003) WNK kinases regulate thiazide-sensitive Na–Cl cotransport. *J Clin Invest* 111:1039–1045