Table A2. (Continued)

Gene name	Allele 1/Allele 2 SNPs	Amino acid change	Region	Allele 1 Homo	Hetero	Allele 2 Homo	Total	Allele frequency Allele 1	Allele frequency Allele 2	Flanking sequence	dbSNP ID
	C39240T[d]		intron25	43	4	0	47	0.957	0.043	gtaagtagtgcc[c/t]ggctggtgggag	rs2289115
	C39375T[e]		intron25	23	20	4	47	0.702	0.298	acatagccctgg[c/t]gattcttagcat	rs2289114
	C48128T	Ile1008Ile	exon26	38	9	0	47	0.904	0.096	agtcatcctgat[c/t]cgaggaaaccag	rs2289113
	A48195G	3′UTR	exon26	46	1	0	47	0.989	0.011	acatccctgtcc[a/g]cagctctgagtg	
MLR	*C−2G*		exon2	0	20	27	47	0.213	0.787	tttatttgttag[c/g]gatggagaccaa	rs2070951
	G218A	Cys73Tyr	exon2	30	1	0	31	0.984	0.016	aactactcccctt[g/a]ccttcagcaaga	rs5522
	G449A	Arg150His	exon2	45	3	0	48	0.969	0.031	gaaatggccatc[g/a]tccttccactct	
	G538A[a]	Val180Ile	exon2	0	14	34	48	0.146	0.854	gtcatgcgcgcc[g/a]ttgttaaaagcc	
	T1497C[a]	Asp499Asp	exon2	0	14	34	48	0.146	0.854	agaaccagatga[t/c]gggagctattac	rs5525
	A1661G	Asn554Ser	exon2	43	5	0	48	0.948	0.052	ttcctcctgtca[a/g]tactttagtgga	rs5527
	G1872A		intron2	45	3	0	48	0.969	0.031	gttttaaggatg[g/a]tcatatgttgct	
WNK1	*G421A*	Ala141Thr	exon1	89	5	0	94	0.973	0.027	cctccagccgct[g/a]ccgcccctgggg	
	C446T	Ala149Val	exon1	90	4	0	94	0.979	0.021	aacaggccgtcg[c/t]gggccctgcccc	
	C511T	Leu171Phe	exon1	93	1	0	94	0.995	0.005	tcccagcctagc[c/t]ttgtggggagca	
	G786A[f]		intron1	0	15	80	95	0.079	0.921	actttatttgac[g/a]gtcctttggatc	rs3858703
	A59884G		intron1	88	1	0	89	0.994	0.006	tctgagttacac[a/g]ttaacagtaaag	
	C73737G[f]		intron3	0	16	79	95	0.084	0.916	gactggctttct[c/g]acattccttttta	rs2158502
	A76571G[f]	Ala429Ala	exon4	0	16	78	94	0.085	0.915	ccaaaatgctgc[a/g]cagatctaccgt	
	C105668A[g]		intron5	91	4	0	95	0.979	0.021	tttctttccct[c/a]tgtttggaagat	
	T105758C[g]	Asp493Asp	exon6	91	4	0	95	0.979	0.021	agcagaagaaga[t/c]gatggagaaaaa	rs2286006
	G105987A		intron6	93	1	0	94	0.995	0.005	tgatgaagtgcc[g/a]tgtgtggcatat	
	A107419G		intron6	75	13	0	88	0.926	0.074	tttcaatatact[a/g]ctgcttaattta	
	C108560T	Thr665Ile	exon8	85	10	0	95	0.947	0.053	cctctgtcttca[c/t]agaatctcgagt	rs2286007
	G124751A[h]	Gln776Gln	exon10	4	26	56	86	0.198	0.802	gccagtgagtca[g/a]cctcaagctcca	rs1012729
	T125972A		intron10	92	1	0	93	0.995	0.005	ttttttttttt[t/a]aagcctgtctgt	
	G126163A[i]	Gln843Gln	exon11	75	20	1	96	0.885	0.115	ccctgtctctca[g/a]attcccatatca	
	A128177C[j]	Thr1056Pro	exon13	3	19	71	93	0.134	0.866	gcagtagcacag[a/c]cccaagctaccc	rs956868
	C128274T[h]		intron13	60	28	5	93	0.796	0.204	gacggtatgaaa[c/t]gccaaactgtca	
	C129494T[i]		intron16	74	20	1	95	0.884	0.116	acaattatggta[c/t]gtctgcatttgg	
	A129852G	Ile1172Met	exon16	88	4	0	92	0.978	0.022	tattctagcaat[a/g]gagagagagtcg	
	C130104T		intron16	90	2	0	92	0.989	0.011	gacacccatgac[c/t]gacaacaaactt	
	T130917G[k]		intron18	44	39	12	95	0.668	0.332	gatattgtagta[t/g]gtgtttatttct	
	C131195T	Asn1320Asn	exon19	20	47	28	95	0.458	0.542	agaaggacccaa[c/t]acagcacctcca	
	C131279T[j]	Thr1348Thr	exon19	72	19	3	94	0.867	0.133	tggagtcccaac[c/t]acagcagcagcc	
	C132236T	Ser1667Ser	exon19	87	2	0	89	0.989	0.011	cagtgaacacag[c/t]tcatctggagct	
	C132444G	Pro1737Ala	exon19	88	1	0	89	0.994	0.006	caagtttctacc[c/g]cagtcagcacta	
	T132576–[j]		intron19	68	17	3	88	0.869	0.131	atcagttttttt[t/-]ctccctaatgag	
	A132655G		intron19	20	36	15	71	0.535	0.465	cttatagtattt[a/g]ttaaattgacag	
	C133634T[i]		intron19	72	19	0	91	0.896	0.104	tttagcgtctca[c/t]ggacttgatttt	
	G135642T[k]	Met1808Ile	exon21	42	42	9	93	0.677	0.323	tagtccagagat[g/t]atcacagtgact	
	T135771G		intron21	92	1	0	93	0.995	0.005	tttaacatgtat[t/g]cagagttcctgc	
	G136943A	Gln1832Gln	exon22	93	1	0	94	0.995	0.005	agcaggaacaca[g/a]cctcagaagggt	
	A141069T	Gly1858Gly	exon23	86	3	0	89	0.983	0.017	ttttaagatggg[a/t]cgatttcaggta	
	C141114T[h]		intron23	58	27	4	89	0.803	0.197	cttgattccttc[c/t]tttggaggagtt	rs2301880
	T142439C[i]		intron23	70	19	1	90	0.883	0.117	tgattctttttt[t/c]ccttttttaaat	
	C142763T	Arg1945Cys	exon24	87	6	0	93	0.968	0.032	accaaggttgga[c/t]gttttcaggtga	
WNK4	*C163T*	Arg55Cys	exon1	95	1	0	96	0.995	0.005	gagccccggccg[c/t]gctcttctcgtc	
	G288A	Arg96Arg	exon1	95	1	0	96	0.995	0.005	tggccccgcgag[g/a]agcccaccgcct	
	C383T	Pro128Leu	exon1	95	1	0	96	0.995	0.005	gtcccgagctcc[c/t]ggactctgcagt	
	T2074C	Ser211Ser	exon2	93	1	0	94	0.995	0.005	tcggaaactgtc[t/c]agagctgagcgg	
	C2285T		intron2	87	7	0	94	0.963	0.037	gatgtgtgccca[c/t]tgcttcctgaac	

Table A2. (Continued)

Gene name	Allele 1/Allele 2 SNPs	Amino acid change	Region	Allele 1 Homo	Hetero	Allele 2 Homo	Total	Allele frequency Allele 1	Allele frequency Allele 2	Flanking sequence	dbSNP ID
	A4732G	Ile474Val	exon6	94	1	0	95	0.995	0.005	gacaaccaggcc[a/g]tcgagttcctgt	
	A6744G	Met546Val	exon7	277	1	0	278	0.998	0.002	gcaactgtgccc[a/g]tggcccccggtc	
	C6749T[l]	Ala567Ala	exon7	87	5	1	93	0.962	0.038	tgtgcccatggc[c/t]cccggtcccccc	
	G7144T	Ala601Ser	exon8	89	6	1	96	0.958	0.042	gcctcagaccct[g/t]cccttcagcccc	
	A7235		intron8	83	12	1	96	0.927	0.073	tgggggctccc[a/del]gccattccaagc	
	G8119A		intron11	95	1	0	96	0.995	0.005	gaggggagaga[g/a]atgaggacagag	
	G12806C[l]		intron12	89	6	1	96	0.958	0.042	cgcgcccagcct[g/c]atgttttaagat	
	T12948C	Ile740Thr	exon12	95	1	0	96	0.995	0.005	ggattcgggaga[t/c]tatccagcgagt	
	G14139C	Gly808Ala	exon14	90	1	0	91	0.995	0.005	catcttctcctg[g/c]aactcctttgtc	
	G14440A[l]	Pro908Pro	exon14	89	6	1	96	0.958	0.042	tttctttcctcc[g/a]tgcccctccact	rs2290042
	C14597T[l]	Pro961Ser	exon14	88	6	1	95	0.958	0.042	cctagtcccctc[c/t]ctagcctgcccc	rs2290041
	C14717T		intron14	75	19	0	94	0.899	0.101	agggagactcca[c/t]tctgcactcttc	rs2290040
	C15503A	Pro1173Thr	exon17	278	1	0	279	0.998	0.002	aagcagcccccca[c/a]cgggtattgtgg	
	T15677C		intron17	275	2	0	277	0.996	0.004	ctgtcgactgtt[t/c]ttctccaggccc	
	C15703T		intron17	277	1	0	278	0.998	0.002	gggggtctgccc[c/t]gggggaatagac	
	C15738A		intron17	272	4	0	276	0.993	0.007	cacctcccctttt[c/a]ctcacttagtgc	
NCX1	*A−23846C*		intron1d	94	1	0	95	0.995	0.005	tcacactgcctt[a/c]aattcagggact	
	T−23690C		intron1d	62	31	2	95	0.816	0.184	aaatttaactta[t/c]agcaaggaaaga	
	C−23449A		intron1d	85	9	1	95	0.942	0.058	catactcacatt[c/a]atgttgaggag	
	T−23200C[m]		intron1d	0	9	86	95	0.047	0.953	attccgcccccct[t/c]tttgttgcggag	rs2301340
	G−23186C[m]		intron1d	0	9	86	95	0.047	0.953	ttgttgcggagg[g/c]aaactgaggttc	rs2301341
	T−23181C		intron1d	18	57	20	95	0.489	0.511	gcggaggcaaac[t/c]gaggttcctgga	rs2301342
	A−22729C		intron1c	71	23	1	95	0.868	0.132	taattatgagga[a/c]agtgatttattg	rs2301343
	A−22660—		intron1c	94	1	0	95	0.995	0.005	gattgctgcatt[a/-]ggtttttccca	
	A−22387C	5′UTR	exon1b	93	3	0	96	0.984	0.016	attaaaaaaaaa[a/c]tcattgatatat	
	C−22144G		intron1b	84	9	2	95	0.932	0.068	gcgcggccacaa[c/g]gcactgcggggc	
	G14A	Arg5Gln	exon2	95	1	0	96	0.995	0.005	tgtacaacatgc[g/a]gcgattaagtct	
	C303T	Ser101Ser	exon2	95	1	0	96	0.995	0.005	tcggttcatgtc[c/t]tctatagaagtc	
	G252581A		intron4	45	40	11	96	0.677	0.323	tcttctctctcc[g/a]tgtctcctact	rs433572
	—255090A		intron5	94	1	0	95	0.995	0.005	tcaggtgataca[-/a]gtagctctgtga	
	C265364T	Arg703Cys	exon9	95	1	0	96	0.995	0.005	gcagaaatgggg[c/t]gccccatcctgg	

dbSNP ID was searched by using SNPper, a CHIP Bioinformatics Tool (Riva and Kohane 2001: http://snpper.chip.org/bio/snpper-enter, as of May 1 of 2003, that was constructed by dbSNP build 112). [a-m]The apparent linkage disequilibrium was indicated in the Gene name column. * Triallelic polymorphism.

151–183.

3. The ALLHAT Officers and Coordinators for ALLHAT Collaborative Research Group: Major outcomes in high-risk hypertensive patients randomised to angiotensin-converting enzyme inhibitor or calcium channel blocker *vs* diuretic. *JAMA* 2002; **288**: 2981–2997.
4. Kaplan NM: Clinical Hypertension. Treatment of Hypertension: Drug Therapy, 8th ed. Philadelphia, Lippincott Williams & Wilkins, 2002.
5. Turner ST, Schwartz GL, Chapman AB, Boerwinkle E: C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. *Hypertension* 2001; **37**: 739–743.
6. Glorioso N, Manunta P, Filigheddu F, *et al*: The role of alpha-adducin polymorphism in blood pressure and sodium handling regulation may not be excluded by a negative association study. *Hypertension* 1999; **34**: 649–654.
7. Sugimoto K, Hozawa A, Katsuya T, *et al*: Alpha-adducin Gly460Trp polymorphism is associated with low renin hypertension in younger subjects in the ohasama study. *J Hypertens* 2002; **20**: 1779–1784.
8. Gitelman HJ, Graham JB, Welt LG: A new familial disorder characterized by hypokalemia and hypomagenesemia. *Trans Assoc Am Physicians* 1966; **79**: 221–235.
9. Simon DB, Nelson WC, Bia MJ, *et al*: Gitleman's variant of Bartter's syndrome, inherited hypokalemic alkalosis, is caused by mutations in the thiazide-sensitive Na-Cl cotrasporter. *Nat Genet* 1996; **12**: 24–30.
10. Wilson FH, Disse-Nicodeme S, Choate KA, *et al*: Human hypertension caused by mutations in WNK kinases. *Science* 2001; **293**: 1107–1112.
11. Geller DS, Rodriguez-Soriano J, Vallo Boado A, *et al*: Mu-

tations in the mineralocorticoid receptor gene cause autosomal dominant pseudohypoaldosteronism type I. *Nat Genet* 1998; **19**: 279–281.
12. Hwang EF, Williams I, Kovacs G, *et al*: Impaired ability of the Na^+/Ca^{2+} exchanger from the Dahl/Rapp salt sensitive rat to regulate cytosolic calcium. *Am J Physiol Renal Physiol* 2003; **284**: F1023–F1031.
13. Okuda T, Fujioka Y, Kamide K, *et al*: Verification of 525 coding SNPs in 179 hypertension candidate genes in the Japanese population: identification of 159 SNPs in 93 genes. *J Hum Genet* 2002; **47**: 387–394.
14. Siffert W, Rosskopf D, Siffert G, *et al*: Association of a human G-protein beta3 subunit variant with hypertension. *Nat Genet* 1998; **18**: 45–48.
15. Nakajima T, Jorde LB, Ishigami T, *et al*: Nucleotide diversity and haplotype structure of the human angiotensinogen gene in two populations. *Am J Hum Genet* 2002; **70**: 108–123.
16. Hilger KF, Delles C, Veelken R, Schmieder RE: Angiotensionogen gene core promoter variants and non-modulating hypertension. *Hypertension* 2001; **38**: 1250–1254.
17. Tanaka C, Kamide K, Takiuchi S, *et al*: An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; **26**: 301–306.
18. Bonnardeaux A, Davies E, Jeunemaitre X, *et al*: Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension* 1994; **24**: 63–69.
19. Lajemi M, Labat C, Gautier S, *et al*: Angiotensin II type 1 receptor −153A/G and 1166A/C gene polymorphisms and increase in aortic stiffness with age in hypertensive subjects. *J Hypertens* 2001; **19**: 407–413.
20. Kupari M, Hautanen A, Lankinen L, *et al*: Association between human aldosterone synthase (CYP11B2) gene polymorphisms and left venyricular size, mass, and function. *Circulation* 1998; **97**: 569–575.
21. Bengtsson K, Melander O, Orho-Melander M, *et al*: Polymorphism in β1-adrenergic receptor gene and hypertension. *Circulation* 2001; **104**: 187–190.
22. Bengtsson K, Orho-Melander M, Melander O, *et al*: β2-Adrenergic receptor gene variation and hypertension in subjects with type 2 diabetes. *Hypertension* 2001; **37**: 1303–1308.
23. Ranade K, Jorgenson E, Sheu W H-H, *et al*: A polymorphism in β1 adrenergic receptor is associated with resting heart rate. *Am J Hum Genet* 2002; **70**: 935–942.
24. Tonolo G, Melis MG, Secchi G, *et al*: Association of Trp64Arg beta 3-adrenergic-receptor gene polymorphism with essential hypertension in the Sardinian population. *J Hypertens* 1999; **17**: 33–38.
25. Shibata K, Hirasawa A, Moriyama N, Kawabe K, Ogawa S, Tsujimoto G: α1a-Adrenoceptor polymorphism: pharmacological characterization and association with benign prostatic hypertrophy. *Br J Pharmacol* 1996; **118**: 1403–1408.
26. Buscher R, Herrmann V, Ring KM, *et al*: Variability in phenylephrine response and essential hypertension: a search for human α1B-adrenergic receptor polymorphisms. *J Pharmacol Exp Ther* 1999; **291**: 793–798.
27. Michel MC, Plogmann C, Philipp T, Brodde O-E: Functional correlates of α2a-adrenocetor gene polymorphism in the HANE study. *Nephrol Dial Transplant* 1999; **14**: 2657–2663.
28. Ardlie KG, Kruglyak L, Seielstad M: Patterns of linkage disequilibrium in the human genome. *Nat Rev* 2002; **3**: 299–309.
29. Cruz DN, Simon DB, Nelson-Williams C, *et al*: Mutations in the Na-Cl contransporter reduce blood pressure in humans. *Hypertension* 2001; **37**: 1458–1464.
30. Melander O, Orho-Melander M, Bengtsson K, *et al*: Genetic variants of thiazide-sensitive NaCl-cotransporter in Gitelman's syndrome and primary hypertension. *Hypertension* 2000; **36**: 389–394.
31. Wilson FH, Kahle KT, Sabath E, *et al*: 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* 2003; **100**: 680–684.
32. Yang CL, Angell J, Mitchell R, Ellison DH: WNK kinases regulate thiazide-sensitive Na-Cl cotransport. *J Clin Invest* 2003; **111**: 1039–1045.
33. Garbers DL, Dubosis SK: The molecular basis of hypertension. *Annu Rev Biochem* 1999; **68**: 127–155.
34. Sciarrone MT, Stella P, Barlassina C, *et al*: ACE and alpha-adducin polymorphism as markers of individual response to diuretic therapy. *Hypertension* 2003; **41**: 398–403.
35. Intersalt Cooperative Research Group: Intersalt: an international study of electrolyte excretion and blood pressure: results for 24 hour urinary sodium and potassium excretion. *Br Med J* 1988; **297**: 319–328.
36. Clement K, Vaisse C, Manning BSJ, *et al*: Genetic variation in the beta-3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *New Engl J Med* 1995; **333**: 352–354.
37. Urhammer SA, Hansen T, Borch-Johnsen K, Pedersen O: Studies of the synergistic effect of the trp/arg64 polymorphism of the beta-3-adrenergic receptor gene and the −3826 A-G variant of the uncoupling protein-1 gene on features of obesity and insulin resistance in a population-based sample of 379 young Danish subjects. *J Clin Endocrinol Metab* 2000; **85**: 3151–3154.
38. Elbein SC, Hoffman M, Barrett K, *et al*: Role of the beta-3-adrenergic receptor locus in obesity and noninsulin-dependent diabetes among members of Caucasian families with a diabetic sibling pair. *J Clin Endocrinol Metab* 1996; **81**: 4422–4427.
39. Fujisawa T, Ikegami H, Yamato E, *et al*: Trp64Arg mutation of 3-adrenergic receptor in essential hypertension; insulin resistance and the adrenergic system. *Am J Hypertens* 1997; **10**: 101–105.
40. Rissanen J, Kuopusjari J, Pihlajamaki J, *et al*: The Trp64Arg polymorphism of the beta 3-adrenergic receptor gene: lack of association with NIDDM and features of insulin resistance syndrome. *Diabetes Care* 1997; **20**: 1319–1323.
41. Shihara N, Yasuda K, Moritani T, *et al*: The association between Trp64Arg polymorphism of the beta 3-adrenergic receptor and autonomic nervous system activity. *J Clin Endocrinol Metab* 1999; **84**: 1623–1627.

隔月連載
第4回
Morning Hypertension

総論：早朝高血圧管理が予後に及ぼす影響をみる

河野雄平
国立循環器病センター内科 高血圧・腎臓部門

はじめに

早朝に血圧が上昇し心血管事故が多発することは，以前よりよく知られている．また，最近では早朝の血圧値や血圧上昇が，臓器障害や心血管予後に関連することが示され，早朝高血圧管理の重要性が唱えられている．早朝高血圧は外来や検診時の血圧が正常な場合にもしばしば認められ，最近注目されている仮面高血圧もこの形をとることが多い．

早朝高血圧のコントロールにより，心血管予後および生命予後が改善することが期待される．しかし，これを目的とした介入試験はきわめて少ない．それでも過去の降圧治療研究はその有効性を示唆しており，また少数の臨床試験が現在おこなわれている．本稿では，早朝高血圧管理が予後に及ぼす影響について概説し，展望を述べる．

1．早朝高血圧と心血管障害

まず早朝に血圧が上昇し心血管事故が多発することや，早朝の血圧値や血圧上昇が臓器障害や心血管予後に関連することについて簡単に述べる．

1）血圧，心血管事故の日内変動

血圧の日内変動はよく知られている．朝の覚醒とともに血圧は急に上昇し，日中は高く，夜になると血圧はいくらか下がり，睡眠により大きく低下する．血圧の日内変動は交感神経活動の変動にほぼ一致しており，精神および身体活動によるところが大きいが，他の機序も関与している．

高血圧患者は，全体としては正常血圧者と同様の血圧日内変動を示し，正常血圧者とくらべると1日を通して高値を呈する．しかし，夜間降圧が減弱している者（non-dipper）や，朝の著しい血圧上昇（morning surge）を示す場合が少なくない[1]．

脳卒中や心筋梗塞などの心血管事故の発症も，朝に多いことがよく知られている．これらは起床直後から3時間以内が最も多く，夜間は最も少ない．脳卒中についてのメタアナリシスでは，出血性，虚血性脳卒中のいずれも早朝に最も多い[2]．心疾患に関しては，狭心症や心臓突然死も朝に多発する．

2）早朝高血圧と臓器障害，予後

早朝の心血管事故の発症には，交感神経系の活動亢進による血圧上昇の関与が考えられる．交感神経活動はまた，心拍数増加や不整脈，心筋虚血をもたらし，血小板凝集能を高めて血栓形成を促進するようにはたらく．

早朝高血圧と心血管事故との関連を調べた研究は意外に少ないが，Gosse ら[3]はベースラインの起床時収縮期血圧値が追跡期間中の心血管合併症に最も強く関係することを観察している（**表1**）．また最近，Kario ら[4]は血圧の

表 1. 追跡中に心血管合併症をおこした高血圧患者とおこさなかった高血圧患者のベースラインの臨床像

	心血管合併症なし	心血管合併症あり	p
人数	214	23	
男性/女性	140/74	20/3	0.04
年齢（歳）	49±12	57±11	0.002
外来 SBP（mmHg）	159±18	169±17	0.008
外来 DBP（mmHg）	98±10	100±9	NS
24 時間 SBP（mmHg）	133±16	143±14	0.001
24 時間 DBP（mmHg）	87±10	91±9	NS
日中 SBP（mmHg）	138±16	149±15	0.002
日中 DBP（mmHg）	92±11	96±11	NS
夜間 SBP（mmHg）	121±17	129±14	0.03
夜間 DBP（mmHg）	78±12	80±10	NS
起床時 SBP（mmHg）	137±22	156±26	<0.001
起床時 DBP（mmHg）	95±15	100±15	NS
起床時 HR（bpm）	81±15	83±20	NS
自動血圧計装着時 SBP（mmHg）	152±20	160±24	NS
自動血圧計装着時 DBP（mmHg）	100±13	104±18	NS
体重（kg）	73±14	73±10	NS
喫煙者（%）	22%	30%	NS
高脂血症（%）	13%	22%	NS
糖尿病（%）	6%	9%	NS
LVM/H$^{2.7}$	53±15	63±13	NS

SBP：収縮期血圧，DBP：拡張期血圧
（Gosse P *et al*，2001[3]より引用）

morning surge が脳卒中の独立した危険因子であることを報告している．しかし，日内変動からみた場合にどの血圧が最も重要かは明らかではない．血圧の平均値に加えて，夜間降圧の減弱や夜間血圧の高値，血圧変動性の増大なども臓器障害や心血管リスクに関連することが報告されている．

早朝高血圧は，未治療の者や治療中の患者において，しばしば認められる．とくに後者では降圧薬治療の結果として生じることがあり，注意を要する[1]．外来や検診時の血圧が正常で 24 時間血圧や家庭血圧が高い仮面高血圧が最近注目されているが，早朝高血圧を呈していることが多い．仮面高血圧は臓器障害を伴うことが多く，心血管予後が不良であることが報告されている[5][6]．

2．早朝高血圧の管理と予後

早朝高血圧が心血管リスクを高めるのであれば，そのコントロールにより予後の改善が期待できよう．しかし，この問題を検討した臨床試験は少なく，エビデンスは乏しい．

1）過去の高血圧治療試験

早朝高血圧管理が予後に及ぼす影響を調べることを目的とした臨床試験はきわめて少ない．しかし，多くの大規模臨床試験の結果からは，降圧薬による治療が心血管予後および生命予後を改善させることが明らかであり，緩和な降圧より厳格な降圧が，より効果的であることも示されている[7]．これらは早朝高血圧の管理を目的としたものではないが，早朝を含めた高血圧管理の重要性を示唆している．

欧州の Syst-Eur（Systolic Hypertension in Europe）と中国の Syst-China（Systolic Hypertension in China）研究は，高齢者の収縮期高血圧への Ca 拮抗薬の有用性を示したものであるが，これらの研究ではニトレンジピンがおもに夕刻に投与されている（大量の場合は朝夕）[8][9]．これらの研究における降圧治療の予後改善効果は明らかであり（図 1），使用薬剤の性質からみれば夜間から早朝の血圧コントロールが予後改善にはたらいた可能性が考えられる．

2）CONVINCE 試験

CONVINCE（Controlled Onset Verapamil Investigation of Cardiovascular End Points）試験は，夜に服薬

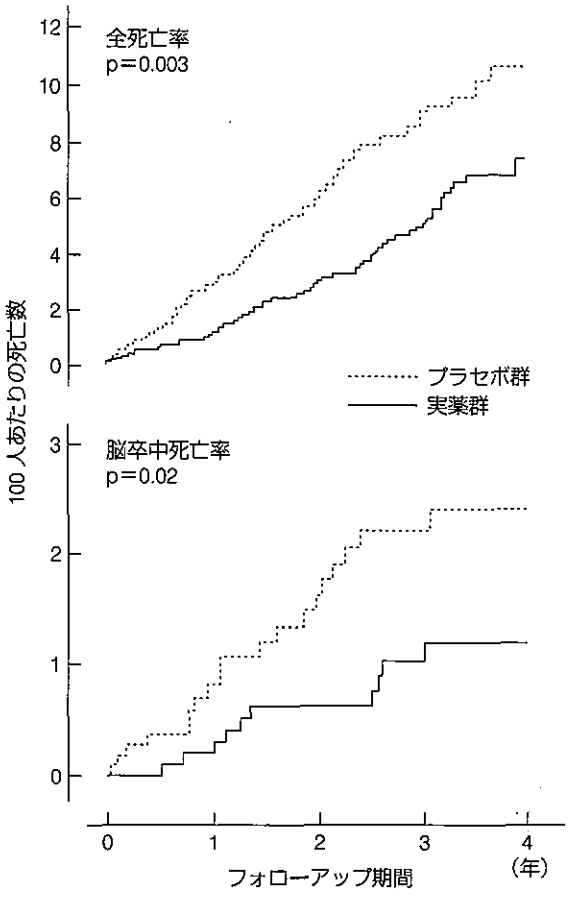


図 1. Syst-China 研究における実薬群とプラセボ群の全死亡率および脳卒中死亡率
(Liu L *et al*, 1998[9]より引用)

すれば早朝に降圧効果が最大となる Ca 拮抗薬ベラパミル製剤とβ遮断薬アテノロールあるいは利尿薬ヒドロクロロチアジドを比較した臨床試験である[10]．これは CORE (controlled-onset slow-release) ベラパミルの心血管疾患予防効果が他剤と同等か否かを検討することを目的としているが，同時に早朝高血圧のコントロールの予後への効果もみた研究であり，心血管事故の発症時刻も調べている．大規模な試験であったが，残念ながらスポンサーの都合で予定より2年早く終了した．

この研究は国際的な多施設共同の無作為二重盲験試験であり，心血管危険因子を有する高血圧患者 16,602 人を対象としている．CORE ベラパミル群（実薬を就寝前，プラセボを早朝服用）とアテノロールあるいはヒドロクロロチアジド群（実薬を早朝，プラセボを就寝前服用）に割り付けられ，血圧コントロールが不十分の場合には他剤が追加された．主要評価項目は脳卒中，心筋梗塞の発症あるいは心血管死亡である．平均追跡期間は3年であった．

結果は，外来血圧は両群とも同等に低下した（CORE ベラパミル群 13.6/7.8 mmHg，対照群 13.5/7.1 mmHg）．主要心血管イベントは CORE ベラパミル群 364 人，対照群 365 人で，同等であった（ハザード比：HR 1.02）．全死亡も有意差はなかった（HR 1.08）．心血管イベントの発症は両群とも午前中（6～12 時）に最も多く，いずれの時間帯にも群間差はみられなかった（図 2）．

CONVINCE 試験の結果は，早朝血圧を目標とした降圧治療は通常の治療とくらべて予後改善効果が優れているわけではないことを示しているようにみえる．しかし，両群の実際の早朝血圧や 24 時間血圧は示されていない．利尿薬は長時間作用型で夜間から早朝の血圧にも効果的で，β遮断薬も早朝血圧を下げることから，両群の早朝血圧に差があったかどうか疑わしい．早朝高血圧管理の有用性については，更なる検討を要すると考えられる．

3）進行中の介入試験

わが国で，早朝の家庭血圧を目標とする2つの無作為介入試験が現在おこなわれている．われわれ[11]の HOSP (Hypertension Control Based On Home Systolic Pressure) 研究と，東北大学今井教授ら[12]による HOMED-BP (Hypertension Objective Treatment based on Measurement by Electrical Devices of Blood Pressure) 研究である．これらは早朝血圧への治療と他の治療法をくらべるものではないが，2つの異なる降圧目標を検討するものであり，早朝高血圧の管理について重要な知見をもたらすことが期待される．2004 年の日本高血圧学会において，それぞれの中間結果が発表された[13][14]．

HOSP 研究は，2000 年にパイロットスタディが開始され，2003 年にメインスタディが開始された．中高年の高血圧患者を対象として，朝の家庭収縮期血圧を 140 mmHg 未満（130 以上）と 130 mmHg 未満の群に，また降圧薬を Ca 拮抗薬アムロジピン群と ARB ロサルタン群に割り付け，5年間治療される．尿アルブミンを調べたサブスタディの1年後の結果は，尿アルブミン排泄量は厳格な降圧群では有意に減少し，緩和な降圧群では不変

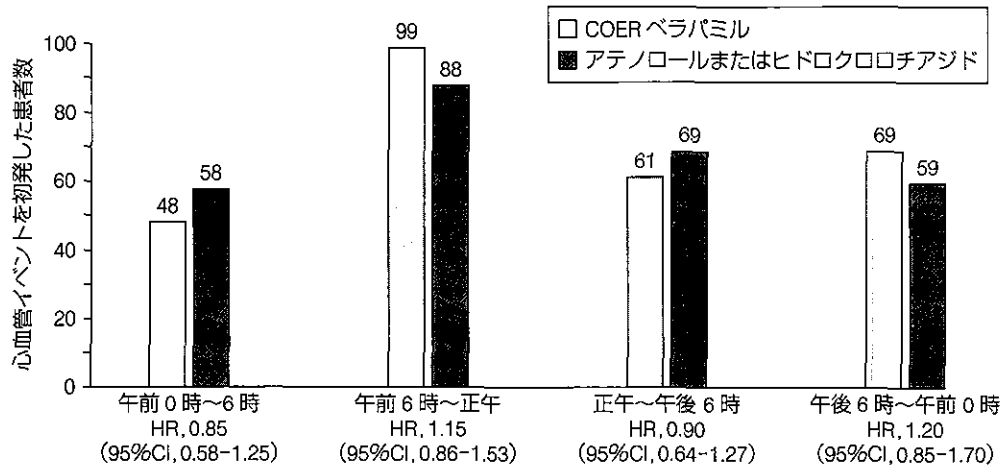


図 2. CONVINCE 試験における COER ベラパミル群とアテノロールまたはヒドロクロロチアジド群の時間別の心血管イベント
(Black HR *et al*, 2003[10]より引用)

表 2. HOSP サブスタディにおける朝の家庭血圧の降圧目標および降圧薬による各群の尿アルブミン排泄量の経過

	尿アルブミン排泄量 (mg/day)		
	治療前	3カ月後	1年後
降圧目標			
140 mmHg 未満	33±37	41±68	36±28
130 mmHg 未満	42±45	38±42	27±34*
降圧薬			
アムロジピン	40±43	36±35	31±25
ロサルタン	36±21	43±69	31±36

*：p<0.05 vs 治療前
(河野雄平ほか, 2002[11]より引用)

であった (**表 2**)[11]. パイロットスタディの 3 年後は，各群とも朝の家庭血圧は目標血圧を達成していた (131/81 および 126/80 mmHg). メインスタディは目標症例数 2,600 人で，心血管イベントを主要評価項目として 2006 年 3 月まで症例登録が進められている[13].

HOMED-BP 研究は，2001 年に開始された．中高年の高血圧患者を対象として，朝の家庭収縮期血圧を 135 mmHg 未満 (125 以上) と 125 mmHg 未満の群に，降圧薬を Ca 拮抗薬群，ACE 阻害薬群，ARB 群に割り付け，7 年間治療される[12]. 目標症例数は 9,000 人であり，すでに 2,700 人以上が登録されている．1 年後の血圧値は高値群 133/79 mmHg，低値群 132/80 mmHg であった[14].

おわりに

早朝血圧が高いことが心血管リスクを高めることは疑いなく，早朝血圧を含めた高血圧管理が心血管予後や生命予後を改善することも確実である．しかし，早朝血圧に目標をしぼった降圧治療が一般的な高血圧治療より予後改善効果が優れているかどうかは，まだ明らかではない．今後の研究の進展を待ちたい．また，早朝血圧の目標をどのレベルに設定し管理すべきかも重要な問題である．現在進行中の臨床研究の結果が期待されるが，当面は家庭血圧の高血圧基準値である 135/85 mmHg より低くなるようにコントロールすることがすすめられる．

文　献

1) 河野雄平ほか：今月の治療 **11**：453, 2003
2) Elliott WJ：*Stroke* **29**：992, 1998
3) Gosse P *et al*：*J Hum Hypertens* **15**：413, 2001
4) Kario K *et al*：*Circulation* **107**：1401, 2003
5) Pickering TG：*Hypertension* **40**：795, 2002
6) Bobrie G *et al*：*JAMA* **291**：1342, 2004
7) Blood Pressure Lowering Treatment Trialists' Collaboration：*Lancet* **362**：1527, 2003
8) Staessen JA *et al*：*Lancet* **350**：757, 1997
9) Liu L *et al*：*J Hypertens* **16**：1823, 1998
10) Black HR *et al*：*JAMA* **289**：2073, 2003
11) 河野雄平ほか：*Ther Res* **23**：790, 2002
12) Fujiwara T *et al*：*Blood Press Monit* **7**：77, 2002
13) 河野雄平ほか：第27回日本高血圧学会プログラム・抄録集：p. 52, 2004
14) 斎藤伸ほか：第27回日本高血圧学会プログラム・抄録集：p. 16, 2004

トピック

仮面高血圧：その診断と治療

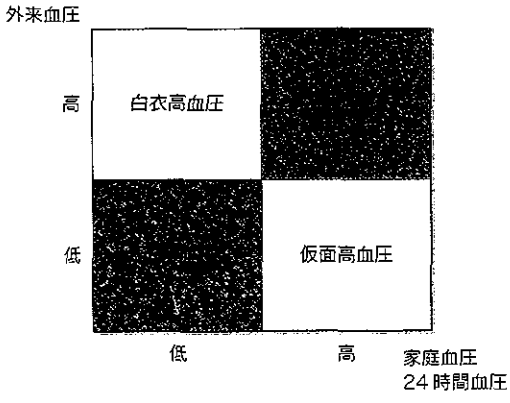
河野雄平 Kawano Yuhei　国立循環器病センター高血圧腎臓内科

検診時や外来受診時に測定された血圧は，日常生活における血圧と大きく異なる場合が少なくない．受診時に高血圧を呈し通常の血圧は正常である白衣高血圧はよく知られているが，最近これとは逆の仮面高血圧（masked hypertension）が臓器障害や予後との関連から注目されている．また，仮面高血圧は治療中の高血圧患者でもしばしば認められる．

本稿では，仮面高血圧の診断と治療について，これまでに得られたエビデンスを含めて概説する．

仮面高血圧の概念と診断

仮面高血圧とは，検診や外来での随時血圧は正常で，24時間血圧や家庭血圧は高値を呈する病態である（❶）．この用語は，Pickeringらによるが[1]，同じ概念は以前より逆白衣高血圧（reverse white-coat hypertension）や白衣正常血圧（white-coat normotension）とよばれていた．また最近では，



❶ 外来血圧と家庭あるいは24時間血圧による血圧分類

孤立性自由行動下高血圧（isolated ambulatory hypertension）ともよばれる．仮面高血圧の呼称は，本来は未治療の者におけるものであるが，降圧治療中の患者においても用いられている．

仮面高血圧の頻度は，対象や診断基準により異なるが，外来血圧が正常な未治療者の10～30％程度と報告されている[2]（Level 3）．すなわち，検診で正常とされる者の少なくとも10％は仮面高血圧であると考えられる．降圧治療中の者における仮面高血圧の頻度も10～30％であり[2]（Level 3），治療中の高血圧患者の20％近くは外来血圧が正常でも24時間血圧や家庭血圧は高いことになる．

仮面高血圧の診断は，24時間血圧あるいは家庭血圧の測定なしではなされえない．実際の診療においては，自由行動下24時間血圧測定（ABPM）はあまり実用的ではなく，家庭血圧測定で十分と考えられる．診断基準は，随時血圧が140/90 mmHg未満で，家庭血圧あるいはABPMでの日中血圧が135/85 mmHg以上としてよいであろう．24時間血圧では，125/80 mmHg以上や135/85 mmHg以上とされている．家庭血圧を用いる場合には，繰り返しの測定により診断する必要がある．

仮面高血圧の病態と予後

仮面高血圧の病態は解明されているわけではないが，いくつかの機序が考えられる[3]．早朝高血圧を呈する者は多く，飲酒などの生活習慣や降圧薬との関係がその機序として考えられる．日中の血圧が高

いが受診時には低い場合は，ストレスや喫煙，身体活動などの関与が疑われる．夜間の高血圧を示すこともあり，睡眠時無呼吸や自律神経障害，降圧薬などによる．仮面高血圧者の臨床的特徴もそれほど明らかではないが，❷に示す項目があげられる．特に，受診時の血圧は低いのに高血圧性の臓器障害を有する場合には，その可能性を疑うべきであろう．

仮面高血圧と臓器障害や予後との関連が，最近明らかになってきた．Pickeringのグループの未治療者における検討では，ABPMにより診断された仮面高血圧者は，心肥大や頸動脈プラークに関して正常血圧者とは異なり，持続性高血圧者と同等であった[4]（❸）．イタリアのPAMELA研究においても，心肥大について類似の成績が示されている（Level 3）．われわれの治療中の高血圧患者における検討でも，仮面高血圧群の心重量，頸動脈壁厚，尿アルブミンは持続性高血圧群よりむしろ高値であった[5]．

仮面高血圧と予後については，未治療の高齢者においてABPMにより診断された仮面高血圧者は，約8年の追跡期間中の心血管イベントは正常血圧者より多く，持続性高血圧者に近いことが報告された[6]（Level 3）．治療中の高血圧患者においても，ABPMによる仮面高血圧群は正常血圧群に比べて心血管イベントが多くなっている[7]（❹）．また，降圧治療中の高齢者において，家庭血圧による仮面高血圧群の心血管イベントは多く，持続性高血圧群をむしろ上回っていた[8]（Level 3）．

❷ 仮面高血圧者の臨床的特徴

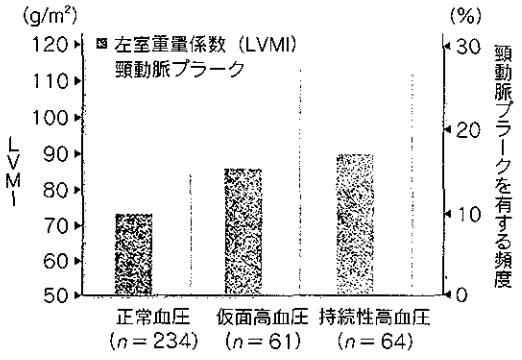
1. 頻度：一般集団や高血圧患者の10～20%
2. 比較的若年者に多い*
3. 男性の割合が多い*
4. 喫煙者または喫煙歴をもつ者が多い
5. アルコール摂取量が多い*
6. 肥満者が多い
7. 代謝障害（耐糖能異常，高脂血症，インスリン抵抗性）を有する率が高い
8. 日中の身体的活動，ストレスとの関係
9. 降圧薬服用との関係
10. 臓器障害，心血管合併症との関係

*：相反する報告もあり．

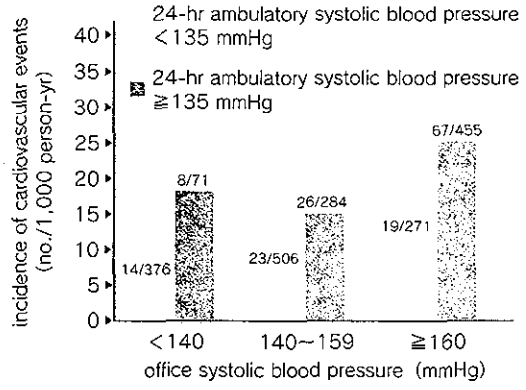
仮面高血圧の治療

前述したように，仮面高血圧は臓器障害を伴うことが多く予後不良であり，診断と治療はきわめて重要と考えられる．ただし，仮面高血圧が問題となったのは最近であり，その治療効果についての無作為臨床試験によるエビデンスはまだ得られていない．

仮面高血圧の治療においても，生活習慣の改善は基本となろう．特に，飲酒や喫煙，ストレスは，朝から日中の血圧上昇をきたしやすいことから（Level 1），飲酒制限，禁煙，ストレスへの対応が望まれる



❸ 仮面高血圧者の臓器障害
(Liu JE, et al. Ann Intern Med 1999; 131: 564-572[4] より作図)



❹ 治療中の高血圧患者の外来および24時間血圧と心血管予後
左から2番目の棒グラフが仮面高血圧群となる．
(Clement DL, et al. N Engl J Med 2003; 348: 2407-2415[7] より)

(❺)[9,10]．また，食塩の過剰摂取は夜間血圧を上昇させることから，食塩制限も重要と考えられる．

仮面高血圧への薬物治療については，24時間の血圧コントロールを考慮した薬剤選択が重要となる．まず，持続性の降圧薬の使用が基本で，作用時間がやや短い薬剤を用いていた場合には変更を考慮する[10]（❺）．Ca拮抗薬やAⅡ受容体拮抗薬，ACE阻害薬などは，半減期の長い薬剤が夜間から早朝の血圧コントロールに優れている（Level 1）．

早朝高血圧を呈する場合には，降圧薬を夜に，あるいは朝と夜に服用するのも効果的であろう．Syst-EurおよびSyst-China試験では，Ca拮抗薬ニトレンジピンの夜または朝夜の服薬により，心血管疾患や痴呆が予防されている（Level 1）．

モーニング・サージが著明な例やストレスが関与する例では，交感神経系の抑制薬がよい適応となろう．前者に対しては，α遮断薬やα β遮断薬あるいは中枢性の交感神経抑制薬の夜の投薬が勧められる（Level 2）．後者には，α β遮断薬かβ遮断薬の朝の投与がよいであろう．夜間から早朝に血圧が上昇するタイプには，利尿薬も勧められる．利尿薬は夜間血圧への効果が比較的大きく，non-dipperがdipperとなることが報告されている（Level 2）．これらの方法を単独あるいは組み合わせて用いることにより，仮面高血圧をコントロールすることができると考えられる．ただし，家庭血圧を基準にする場合には，血圧計の精度や測定法に留意を要する．

仮面高血圧の診断と病態，治療について述べた．仮面高血圧者は，検診や外来では正常血圧と判定されるために十分な治療を受けず，臓器障害をきたし予後不良となると考えられる．したがって診断がきわめて重要であり，その頻度を考えれば高血圧者のみでなく正常者も含めた家庭血圧測定の啓蒙と普及が望まれる．仮面高血圧の治療においては，その原因となりうる生活習慣の改善と，作用時間と作用機序を考慮した降圧薬の選択が，ともに重要と考えられる．今後の前向き臨床試験による治療効果のエビデンスが期待される．EBM

❺ 仮面高血圧の治療

①生活習慣に注意（飲酒，喫煙，ストレス）
②持続性を有する降圧薬の使用
③降圧薬を夜に用いる
④交感神経系の抑制（α受容体遮断）
⑤体液量のコントロール（利尿薬の使用）

文献

1) Pickering TG, Davidson K, Gerin W, et al: Masked hypertension. Hypertension 2002; 40: 795-796.
2) 小原　拓，大久保孝義，今井　潤：白衣高血圧，仮面高血圧の定義と頻度．血圧 2004; 11: 783-787.
3) 堀尾武史，河野雄平：仮面高血圧，逆白衣現象の機序と特徴．血圧 2004; 11: 798-801.
4) Liu JE, Roman MJ, Pini R, et al: Cardiac and arterial target organ damage in adults with elevated ambulatory and normal office blood pressure. Ann Intern Med 1999; 131: 564-572.
5) 富山真理，堀尾武史，吉井正義ほか：仮面高血圧と標的臓器障害—治療下本態性高血圧患者における検討．第27回日本高血圧学会総会プログラム・抄録集．2004．p.19.
6) Bjorklund K, Lind L, Zethelius B, et al: Isolated ambulatory hypertension predicts cardiovascular morbidity in elderly men. Circulation 2003; 107: 1297-1302.
7) Clement DL, De Buyzere ML, De Bacquer DA, et al: Prognostic value of ambulatory blood-pressure recordings in patients with treated hypertension. N Engl J Med 2003; 348: 2407-2415.
8) Bobrie G, Chatellier G, Genes N, et al: Cardiovascular prognosis of "masked hypertension" detected by blood pressure self-measurement in elderly treated hypertensive patients. JAMA 2004; 291: 1342-1349.
9) Kawano Y, Omae T: Lifestyle modifications in the management of hypertension: benefits and limitations. CVD Prevention 1998; 1: 336-346.
10) 河野雄平：病院では正常，しかし家庭では高い血圧：白衣正常血圧（逆白衣高血圧）への対応．Modern Physician 2002; 22: 1534-1535.

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ORIGINAL ARTICLE

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

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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* (*PRKWNK1*) and *WNK4* (*PRKWNK4*) 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

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

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* (*PRKWNK1*) and *WNK4* (*PRKWNK4*) 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

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 (Erlich 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 questionnaired 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 ≥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-susceptive 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 ± 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±11.0	66.3±11.1*
Systolic blood pressure (mmHg)	128.0±19.6	131.9±19.5*
Diastolic blood pressure (mmHg)	76.6±9.8	79.7±10.7*
Body mass index (kg/m^2)	22.3±3.2	23.3±3.0*
Total cholesterol (mmol/l)	5.57±0.79*	5.10±0.78
HDL cholesterol (mmol/l)	1.67±0.40*	1.42±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*, *WNK1*, and *WNK4* 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/snpper-enter, 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 WNK4 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		
TSC	*C-1991A*	a		Promoter	38	10	0	48	0.896	0.104	CACCACTGCCTC[C/A]CTGCAATGGCTT	
	A-950G	b		Promoter	1	19	21	41	0.256	0.744	TTTAATAGAGAC[A/G]GGGTTTCACCAT	
	C-704T			Promoter	46	1	0	47	0.989	0.011	CAGACAGCCCGG[C/T]GCCACACCCTGG	
	C-605T	a		Promoter	37	10	0	47	0.894	0.106	CACTTTGAAAAT[C/T]CCTGTCCTGTTT	
	C-553T			Promoter	26	1	0	27	0.981	0.019	AGCCCCCAGTCA[C/T]GTACCCCCTGCT	
	−544delT			Promoter	47	1	0	48	0.990	0.010	TCACGTACCCCC[T/−]GCTTGCTCAATC	
	C-213G	a		Promoter	35	8	0	43	0.907	0.093	GGGAGTGGCTGG[C/G]TTTGGGCCAGCC	
	C-142T	b		Promoter	1	20	22	43	0.256	0.744	GTGTTCTGCCTC[C/T]GGCCCTGTCCGG	
	G-141C	b		Promoter	28	15	0	43	0.826	0.174	TGTTCTGCCTCC[G/C]GCCCTGTCCGGG	
	C1784T			Intron 1	30	17	1	48	0.802	0.198	TGGATGCAGAGA[C/T]GCCGTCCCTAGC	
	A1918G		Ala122Ala	Exon 2	31	17	0	48	0.823	0.177	GGAGGGCGAGGC[A/G]GGCACCAGCAGC	rs2304479
	A2141T			Intron 2	0	8	40	48	0.083	0.917	ACAATAGATTAA[A/T]GCCTGCCGGGGA	rs2304480
	G2971A			Intron 2	47	1	0	48	0.990	0.010	TAGGGCCTAGGT[G/A]CTCGATACCCTG	
	C4527A		Thr180Lys	Exon 4	43	2	0	45	0.978	0.022	TGCTGTCGGTCA[C/A]GGTGACCTCCAT	
	C7479T		Phe341Phe	Exon 8	38	2	0	40	0.975	0.025	TGGCACCTTCTT[C/T]GGAATGTTCTCC	
	C14272T	c		Intron 10	26	18	3	47	0.745	0.255	CTGGCTCAGCCC[C/T]CACCGTGGAGTC	rs3816119
	G14277A			Intron 10	46	1	0	47	0.989	0.011	TCAGCCCCCACC[G/A]TGGAGTCCCTGA	
	C14363A		Ala464Ala	Exon 11	45	2	0	47	0.979	0.021	CATCTTCGGGGC[C/A]ACCCTCTCCTCT	
	C14366T		Thr465Thr	Exon 11	46	1	0	47	0.989	0.011	CTTCGGGGCCAC[C/T]CTCTCCTCTGCC	rs5801
	G17337A			Intron 13	44	1	0	45	0.989	0.011	GGGGTGGGAGTG[G/A]GAGGCATGGGTG	
	T18806C	d		Intron 13	6	24	18	48	0.375	0.625	GACTGGTGCCCT[T/C]GGCCCAGGGTGG	rs2304483
	C18850T		Ala569Val	Exon 14	46	2	0	48	0.979	0.021	ACAACAAGTGGG[C/T]GGCGCTGTTTGG	
	T20072C		Leu623Pro	Exon 15	46	1	0	47	0.989	0.011	GCTCCTACAACC[T/C]GGCCCTCAGCTA	
	G20088A		Ser628Ser	Exon 15	46	1	0	47	0.989	0.011	CCTCAGCTACTC[G/A]GTGGGCCTCAAT	
	C20201G			Intron 15	46	1	0	47	0.989	0.011	GAGTTTCCAAGC[C/G]TAGACCTGTCAC	
	G21421A	e		Intron 16	20	24	3	47	0.681	0.319	ATGGGGGCCCAA[G/A]GGGATGCGGAGC	
	C21500T			Intron 16	42	2	0	44	0.977	0.023	CCCTCTTGCTGG[C/T]TTCTCCCCCAGC	
	C21566G			Intron 16	43	1	0	44	0.989	0.011	CACTTTCTCCCC[C/G]ACTCCTTGTGTT	
	A21586G			Intron 16	43	1	0	44	0.989	0.011	GTGTTTTCCCTT[A/G]TCTGGGCAAAAG	
	C21822T	c	Ala714Ala	Exon 17	21	21	3	45	0.700	0.300	GGATGTCATTGC[C/T]GAGGACCTCCGC	
	C22682T			Intron 17	46	1	0	47	0.989	0.011	TCACCCCTATCC[C/T]CTGGCAGGCCGC	
	C25013T	c		Intron 18	23	22	3	48	0.708	0.292	CTGGGGGAGAAG[C/T]TGGACCTCACCT	rs3764264
	G27029A	c		Intron 20	18	25	4	47	0.649	0.351	TTTTCTTGTGAC[G/A]GTGGTGCCTGAG	
	C27646T	d		Intron 20	6	26	15	47	0.404	0.596	AAGGGGCGTTGG[C/T]GGGGCCCTGGGC	rs2278490
	T27681C[a]	d		Intron 20	5	23	18	47	0.351	0.628	TGGATGCGCGGC[T/C]GCTGGCTCTGCT	rs2278489
	A27681C[a]				0	1			0.011		TGGATGCGCGGC[A/C]GCTGGCTCTGCT	
	T27681A[a]					0					TGGATGCGCGGC[T/A]GCTGGCTCTGCT	
	T29320A		Leu849His	Exon 22	367	5	0	372	0.993	0.007	TCATTCCCTATC[T/A]CCTTGGCCGCAA	
	C29372T	c	Gly866Gly	Exon 22	23	22	3	48	0.708	0.292	TGTGTTCGTAGG[C/T]GGCCAGATTAAC	rs5804
	G34262A	f		Intron 22	44	1	3	48	0.927	0.073	TCTCAAGAAAAA[G/A]TAATAACAATAA	
	G34372A	g	Arg904Gln	Exon 23	45	3	0	48	0.969	0.031	ACCAGAACCCTC[G/A]GGCTGAGCAGTA	
	C34588T	f		Intron 23	41	3	4	48	0.885	0.115	CACAGGGCAAGG[C/T]GGCTGCAGCCCC	
	T37125C			Intron 23	46	1	0	47	0.989	0.011	CCTCAACCCACT[T/C]TCTCGTCCCCAG	
	C37210T		Asn931Asn	Exon 24	46	1	0	47	0.989	0.011	GGCCACTGTCAA[C/T]GAGATGCGGCGG	

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 frequency Allele 2	Flanking sequence	dbSNP ID
	A37311G	e		Intron 24	23	21	3	47	0.713	0.287	ACGCGACACATC[A/G]CTGGGTCAGGGA	rs2289117
	G39097A			Intron 24	29	1	0	30	0.983	0.017	GAGGCCATAGAC[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]GGCTTTCTCCCG	rs3816118
	G39143A	g		Intron 24	44	3	0	47	0.968	0.032	AAGTGACCACTC[G/A]GCTTTCTCCCGC	rs2289116
	C39203T		Ser967Phe	Exon 25	46	1	0	47	0.989	0.011	TGCTGGATTACT[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]GATTCTTAGCAT	rs2289114
	C48128T		Ile1008Ile	Exon 26	38	9	0	47	0.904	0.096	AGTCATCCTGAT[C/T]CGAGGAAACCAG	rs2289113
	A48195G		3'UTR	Exon 26	46	1	0	47	0.989	0.011	ACATCCCTGTCC[A/G]CAGCTCTGAGTG	
WNK1	*G421A*		Ala141Thr	Exon 1	89	5	0	94	0.973	0.027	CCTCCAGCCGCT[G/A]CCGCCCCTGGGG	
	C446T		Ala149Val	Exon 1	90	4	0	94	0.979	0.021	AACAGGCCGTCG[C/T]GGGCCCTGCCCC	
	C511T		*Leu171Phe*	Exon 1	93	1	0	94	0.995	0.005	TCCCAGCCTAGC[C/T]TTGTGGGGAGCA	
	G786A	a,b,c		Intron 1	0	15	80	95	0.079	0.921	ACTTTATTTGAC[G/A]GTCCTTTGGATC	rs3858703
	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	GACTGGCTTTCT[C/G]ACATTCCTTTTA	rs2158502
	A76571G	a,b	Ala429Ala	Exon 4	0	16	78	94	0.085	0.915	CCAAAATGCTGC[A/G]CAGATCTACCGT	
	C105668A	d		Intron 5	91	4	0	95	0.979	0.021	TTTCTTTTCCCT[C/A]TGTTTGGAAGAT	
	T105758C	d	Asp493Asp	Exon 6	91	4	0	95	0.979	0.021	AGCAGAAGAAGA[T/C]GATGGAGAAAAA	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]CTGCTTAATTTA	
	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	TTTTTTTTTTTT[T/A]AAGCCTGTCTGT	
	G126163A	h	Gln843Gln	Exon 11	75	20	1	96	0.885	0.115	CCCTGTCTCTCA[G/A]ATTCCCATATCA	
	A128177C	i	Thr1056Pro	Exon 13	3	19	71	93	0.134	0.866	GCAGTAGCACAG[A/C]CCCAAGCTACCC	rs956868
	C128274T	e,f,g		Intron 13	60	28	5	93	0.796	0.204	GACGGTATGAAA[C/T]GCCAAACTGTCA	
	C129494T	h		Intron 16	74	20	1	95	0.884	0.116	ACAATTATGGTA[C/T]GTCTGCATTTGG	
	A129852G		Ile1172Met	Exon 16	88	4	0	92	0.978	0.022	TATTCTAGCAAT[A/G]GAGAGAGAGTCG	
	C130104T			Intron 16	90	2	0	92	0.989	0.011	GACACCCATGAC[C/T]GACAACAAACTT	
	T130917G	e,f,g,j		Intron 18	44	39	12	95	0.668	0.332	GATATTGTAGTA[T/G]GTGTTTATTTCT	
	C131195T	f,g,j,k	Asn1320Asn	Exon 19	20	47	28	95	0.458	0.542	AGAAGGACCCAA[C/T]ACAGCACCTCCA	
	C131279T	i	*Thr1348Thr*	Exon 19	72	19	3	94	0.867	0.133	TGGAGTCCCAAC[C/T]ACAGCAGCAGCC	
	C132236T		Ser1667Ser	Exon 19	87	2	0	89	0.989	0.011	CAGTGAACACAG[C/T]TCATCTGGAGCT	
	C132444G		Pro1737Ala	Exon 19	88	1	0	89	0.994	0.006	CAAGTTTCTACC[C/G]CAGTCAGCACTA	
	132576delT	i		Intron 19	68	17	3	88	0.869	0.131	ATCAGTTTTTTT[T/−]CTCCCTAATGAG	
	A132655G	g,j,k		Intron 19	20	36	15	71	0.535	0.465	CTTATAGTATTT[A/G]TTAAATTGACAG	
	C133634T	h		Intron 19	72	19	0	91	0.896	0.104	TTTAGCGTCTCA[C/T]GGACTTGATTTT	
	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	TTTAACATGTAT[T/G]CAGAGTTCCTGC	
	G136943A		Gln1832Gln	Exon 22	93	1	0	94	0.995	0.005	AGCAGGAACACA[G/A]CCTCAGAAGGGT	
	A141069T		Gly1858Gly	Exon 23	86	3	0	89	0.983	0.017	TTTTAAGATGGG[A/T]CGATTTCAGGTA	
	C141114T	e,f,g		Intron 23	58	27	4	89	0.803	0.197	CTTGATTCCTTC[C/T]TTTGGAGGAGTT	rs2301880
	T142439C	h		Intron 23	70	19	1	90	0.883	0.117	TGATTCTTTTTT[T/C]CCTTTTTTAAAT	
	C142763T		Arg1945Cys	Exon 24	87	6	0	93	0.968	0.032	ACCAAGGTTGGA[C/T]GTTTTCAGGTGA	
WNK4	*C163T*		Arg55Cys	Exon 1	95	1	0	96	0.995	0.005	GAGCCCCGGCCG[C/T]GCTCTTCTCGTC	
	G288A		Arg96Arg	Exon 1	95	1	0	96	0.995	0.005	TGGCCCCGCGAG[G/A]AGCCCACCGCCT	
	C383T		Pro128Leu	Exon 1	95	1	0	96	0.995	0.005	GTCCCGAGCTCC[C/T]GGACTCTGCAGT	

T2074C	Ser211Ser	Exon 2	93	1	0	94	0.995	0.005	TCGGAAACTGTC[T/C]AGAGCTGAGCGG
C2285T		Intron 2	87	7	0	94	0.963	0.037	GATGTGTGCCCA[C/T]TGCTTCCTGAAC
A4732G	Ile474Val	Exon 6	94	1	0	95	0.995	0.005	GACAACCAGGCC[A/G]TCGAGTTCCTGT
A6744G	Met546Val	Exon 7	277	1	0	278	0.998	0.002	GCAACTGTGCCC[A/G]TGGCCCCCGGTC
C6749T	Ala567Ala	Exon 7	87	5	1	93	0.962	0.038	TGTGCCCATGGC[C/T]CCCGGTCCCCCC
G7144T	Ala601Ser	Exon 8	89	6	1	96	0.958	0.042	GCCTCAGACCCT[G/T]CCCTTCAGCCCC
A7235		Intron 8	83	12	1	96	0.927	0.073	TGGGGGGCTCCC[A/DEL]GCCATTCCAAGC
G8119A		Intron 11	95	1	0	96	0.995	0.005	GAGGGGGAGAGA[G/A]ATGAGGACAGAG
G12806C		Intron 12	89	6	1	96	0.958	0.042	CGCGCCCAGCCT[G/C]ATGTTTTAAGAT
T12948C	Ile740Thr	Exon 12	95	1	0	96	0.995	0.005	GGATTCGGGAGA[T/C]TATCCAGCGAGT
G14139C	Gly808Ala	Exon 14	90	1	0	91	0.995	0.005	CATCTTCTCCTG[G/C]AACTCCTTTGTC
G14440A	Pro908Pro	Exon 14	89	6	1	96	0.958	0.042	TTTCTTTCCTCC[G/A]TGCCCCTCCACT
C14597T	Pro961Ser	Exon 14	88	6	1	95	0.958	0.042	CCTAGTCCCCCTC[C/T]CTAGCCTGCCCC
C14717T		Intron 14	75	19	0	94	0.899	0.101	AGGGAGACTCCA[C/T]TCTGCACTCTTC
C15503A	Pro1173Thr	Exon 17	278	1	0	279	0.998	0.002	AAGCAGCCCCCA[C/A]CGGGTATTGTGG
T15677C		Intron 17	275	2	0	277	0.996	0.004	CTGTCGACTGTT[T/C]TTCTCCAGGCCC
C15703T		Intron 17	277	1	0	278	0.998	0.002	GGGGGTCTGCCC[C/T]GGGGGAATAGAC
C15738A		Intron 17	272	4	0	276	0.993	0.007	CACCTCCCCTTT[C/A]CTCACTTAGTGC

[a]Triallelic polymorphism

2004). The method of direct sequencing was described previously (Okuda et al. 2002). The polymorphisms were identified by use of Sequencher 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
TSC	*C-1991A*	CCCTGACAGCTCAAATTTCCAC	Fam-CTGCCTCCCTGCAA-MGB
		CTTGTTACCAGAGGTGCCTAAGC	Vic-CTGCCTCACTGCAA-MGB
	C-605T	GCAGAAATGAAATCCACAAGCA	Fam-TTTGAAAATCCCTGTCCTG-MGB
		CATGCACCGATCATTAGATTGG	Vic-CTTTGAAAATTCCTGTCCTG-MGB
	C-213G	GGCAGAACACCATTTGATTGTG	Fam-CTGGCCCAAAGCCAGCCACTC-TAMRA
		GAAGAGCCACTCCAGGACTCA	Vic-CTGGCCCAAACCCAGCCACTC-TAMRA
	C1784T	CGCAGTGGTGCAGGTCAGT	Fam-CAGAGACGCCGTCC-MGB
		AGGTGTCTGCCTTCCTGCTG	Vic-TGCAGAGATGCCGTC-MGB
	A1918G	CTCACCATCACCCCCTTGAC	Fam-CTGGTGCCTGCCTCGCCC-TAMRA
		CAGCAGGAAGGCAGACACCT	Vic-TGGTGCCCGCCTCGCC-TAMRA
	A2141T	GCTTCAGTTTCCCCATCTGTACA	Fam-AATAGATTAAAGCCTGCCGG-MGB
		GGTGGCTTTTTAGGGAAACACA	Vic-AATAGATTAATGCCTGCCGG-MGB
	C4527A	GATGAACGTAGGTCGCATGGT	Fam-TGTCGGTCACGGTGA-MGB
		GATGGCTGAGATGGAGAGGC	Vic-TGTCGGTCAAGGTG-MGB
	T18806C	AGCAGCTCTGGCCTAGAAAGAG	Fam-TGGTGCCCTTGGCCCAGG-TAMRA
		ACGGAGATGATAGCCCCAAAC	Vic-CTGGTGCCCTCGGCCCAG-TAMRA
	T29320A	TCACATAGTGCTCTGTCCTGAGTG	Fam-TCCCTATCTCCTTGGC-MGB
		GATCTTGCATTTGCTCCACCTC	Vic-CCTATCACCTTGGCC-MGB
	C29372T	GCAAGAGGAGGTGGAGCAAAT	Fam-TTCGTAGGCGGCCAG-MGB
		CCCTCCACACTTACGCCTTTC	Vic-TCGTAGGTGGCCAGAT-MGB
	G34372A	GGGATTCCATGAAGTCCACATC	Fam-AACCCTCGGGCTGA-MGB
		CTGGAAGCCCCAAAACAGAAC	Vic-AGAACCCTCAGGCTG-MGB
	C39375T	GAAGCAGAAGGGCCAAAGTTC	Fam-ATAGCCCTGGCGATT-MGB
		GATGCCTGGGACACGTGAG	Vic-TAGCCCTGGTGATTC-MGB
WNK1	*G786A*	GAACTGCAGGTAAAGCCCCAC	Fam-TTTGACGGTCCTTTG-MGB
		GAACTCGATCAACTGGCTTCG	Vic-TTTATTTGACAGTCCTTTG-MGB
	C108560T	CTGATGGGACGGTTGACAGTG	Fam-TCTTCACAGAATCTCGA-MGB
		CCTGTTCATGTTGGGAACCATA	Vic-TCTTCATAGAATCTCG-MGB
	A128177C	GTTGCTCCTGCAGAGCCAGT	Fam-AGTAGCACAGACCCAA-MGB
		TCTACAGAGGAAGCCAAAGTGGT	Vic-AGTAGCACAGCCCCA-MGB
	C133634T	TTGATTTGCTCTTCAGTACGCAG	Fam-AGCGTCTCACGGACT-MGB
		GCACCTACAGACAACAAAGGGAA	Vic-AGCGTCTCATGGACT-MGB
	G135642T	AAAACTTACACCAACCGCAGAAG	Fam-CTGTGATCATCTCTG-MGB
		ATTCAGTCCCAGCAACCTCTAGA	Vic-ACTGTGATAATCTCTG-MGB
	C141114T	TGGGACGATTTCAGGTAAGACAG	Fam-ATTCCTTCCTTTGGAGGA-MGB
		TTGTGTCCCAAATAGGTAGGCA	Vic-ATTCCTTCTTTTGGAGGAG-MGB
	C142763T	ACGACCCACTTTGTTTGCTGTA	Fam-CTGAAAACGTCCAACCT-MGB
		GTCAGACACTGGGCAGCCTAC	Vic-CCTGAAAACATCCAACCT-MGB
WNK4	*C14597T*	CTGGCTGTGATGACTGTGGC	Fam-TCCCCTCCCTAGCCT-MGB
		TGAAGGGCTTTCCTGGCC	Vic-TCCCCTCTCTAGCCTG-MGB
	C14717T	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 ($n=983$)				Men ($n=835$)			
			Normotensive ($n=608$)	Hypertensive ($n=375$)	χ^2	p	Normotensive ($n=439$)	Hypertensive ($n=396$)	χ^2	p
TSC	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
TSC	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
TSC	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
TSC	C1784T	(CC/CT/TT)	435/161/12	289/81/5	3.754	0.153	320/112/7	293/94/9	0.800	0.670
TSC	A1918G	(AA/AG/GG)	407/175/26	240/118/17	0.900	0.638	283/133/23	253/131/12	2.945	0.229
TSC	A2141T	(AA/AT/TT)	6/114/488	4/67/304	0.131	0.936	2/85/352	3/71/322	0.579	0.749
TSC	C4527A	(CC/CA/AA)	591/17/0	362/13/0	*n.d.*	*n.d.*	427/12/0	382/14/0	*n.d.*	*n.d.*
TSC	T18806C	(TT/TC/CC)	115/285/208	63/181/131	0.703	0.704	57/210/172	50/182/164	0.435	0.804
TSC	T29320A	(TT/TA/AA)	592/16/0	360/15/0	*n.d.*	*n.d.*	428/11/0	391/5/0	*n.d.*	*n.d.*
TSC	C29372T	(CC/CT/TT)	325/242/41	199/143/33	1.475	0.478	36/186/36	213/155/28	1.645	0.439
TSC	G34372A	(GG/GA/AA)	548/59/1	334/40/1	0.362	0.835	387/50/2	347/49/0	*n.d.*	*n.d.*
TSC	C39375T	(CC/CT/TT)	342/222/44	207/146/22	1.057	0.589	231/174/34	189/161/46	4.302	0.116
WNK1	G786A	(GG/GA/AA)	9/133/466	4/93/278	1.356	0.508	7/82/350	7/82/307	0.602	0.740
WNK1	C108560T	(CC/CT/TT)	527/76/5	310/62/3	3.127	0.209	377/60/2	342/52/2	0.061	0.970
WNK1	A128177C	(AA/AC/CC)	9/135/464	4/80/291	0.430	0.807	9/102/328	8/86/302	0.280	0.869
WNK1	C133634T	(CC/CT/TT)	453/143/12	267/101/7	1.449	0.485	335/94/10	296/93/7	0.733	0.693
WNK1	G135642T	(GG/GT/TT)	244/290/74	139/182/54	1.478	0.478	196/187/56	164/182/50	1.040	0.595
WNK1	C141114T	(CC/CT/TT)	361/218/29	219/134/22	0.576	0.750	278/135/26	241/134/21	0.962	0.618
WNK1	C142763T	(CC/CT/TT)	592/16/0	362/13/0	*n.d.*	*n.d.*	421/17/1	389/7/0	*n.d.*	*n.d.*
WNK4	C14597T	(CC/CT/TT)	581/27/0	353/22/0	*n.d.*	*n.d.*	410/29/0	375/21/0	*n.d.*	*n.d.*
WNK4	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 ± SDs; all adjusted for age, body mass index (BMI), antihypertensive drug use, present illness (hyperlipidemia, diabetes mellitus), and lifestyle (smoking and drinking); diastolic blood pressure (DBP) and systolic blood pressure (SBP) are expressed as mmHg

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

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)	*p*	Genotype	OR[a] (95% CI)	*p*
Men	CC	1 (reference)		CC+CT	1 (reference)	
	CT+TT	1.62 (1.12–2.33)	0.010	TT	3.00(0.88–10.19)	0.079
Women	CC	1 (reference)		CC+CT	1 (reference)	
	CT+TT	0.80(0.56–1.13)	0.209	TT	0.74 (0.22–2.45)	0.621

[a]Conditional 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.

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References

Baima J, Nicolaou M, Schwartz F, DeStefano AL, Manolis A, Gavras I, Laffer C, Elijovich 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

Benjafield 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, Ogihara 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, Gitleman HJ, Lifton RP (1996) Gitelman's variant of Bartter's syndrome, inherited hypokalaemic 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, Ogihara T, Tomoike H (2004) Indentification 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