Original Article

The Thiazide-Sensitive Na^+-Cl^- Cotransporter Gene, *C1784T*, and Adrenergic Receptor-β3 Gene, *T727C*, May Be Gene Polymorphisms Susceptible to the Antihypertensive Effect of Thiazide Diuretics

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The response of blood pressure to thiazide diuretics (TZDs) differs among individuals. The prediction of the antihypertensive effect of TZDs is important for realizing individualized therapy in the management of hypertension. The aim of this study was to identify the single nucleotide polymorphisms (SNPs) susceptible to the antihypertensive effect of TZDs, particularly focusing on genes related to water-electrolyte absorption in the kidney. Seventy-six outpatients (mean age, 65.4±9.0 years) with essential hypertension (EHT) taking TZDs were retrospectively assessed. We defined as responders (R) those whose mean blood pressure was lowered by more than 5 mmHg after the use of TZDs. Forty-eight SNPs in 17 genes (*ADD1*, *GNB3*, *TSC* [*SLC12A3*], *MLR* [*NR3C2*], *NCX1* [*SLC8A1*], *WNK1*, *WNK4*, *AGT*, *ACE*, *AT1* [*AGTR1*], *CYP11B2*, *ADRB1*, *ADRB2*, *ADRB3*, *ADRA1A*, *ADRA1B*, *ADRA2A*) were genotyped in the 76 patients. The SNPs in *TSC*, *MLR*, *NCX1*, *WNK1*, and *WNK4* were identified by direct sequencing and those with minor frequencies of greater than 5% were genotyped in this study. The comparison of polymorphism prevalence between R and non-responders (NR) showed significant differences in *TSC C1784T* (*C* allele *vs.* *T* allele, odds ratio (OR)=3.81, p=0.016, confidence interval (CI): 1.25–11.63) and *ADRB3 T727C* (*Trp64Arg*) (*T* allele *vs.* *C* allele, OR=4.59, p=0.005, CI: 1.54–13.68). The blood pressure (BP) in patients homozygous for the major alleles of both *TSC C1784T* and *ADRB3 T727C* were significantly reduced by TZD treatment; however, the BP in those homozygous for the minor allele and heterozygous (*TSC C1784T*: TT+CT; *ADRB3 T727C*: CC+CT) for both SNPs were not significantly changed after TZD treatment. Both newly detected *TSC C1784T* and *ADRB3 T727C* are gene polymorphisms susceptible to the antihypertensive effect of TZDs in patients with EHT. Thus, the prediction of BP reduction by TZDs may be possible by evaluating these two SNPs.
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***Key Words*: thiazide diuretics, gene polymorphism, essential hypertension**

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Introduction

Thiazide diuretics (TZDs) have been most widely used as a first line antihypertensive drug (*1*, *2*). Recently, the ALLHAT study confirmed the usefulness of TZDs for the reduction of blood pressure (BP) and cardiovascular diseases in comparison with newer antihypertensive drugs, including Ca channel blockers (CCBs) such as amlodipine and angiotensin converting enzyme inhibitors (ACEIs) such as lisinopril in about 40,000 hypertensive patients with high risk factors (*3*). TZDs are not only effective as a monotherapy for hypertension, but are also very useful for combination therapy with other antihypertensive drugs (*4*). Moreover, the use of a TZD as a drug therapy for hypertension, which is a chronic and lifelong disease, would be very good from the viewpoint of the cost of drugs, because TZDs are the cheapest of all antihypertensive drugs. However, the response of BP to TZDs differs among individuals, and TZDs often induce side effects, such as hypokalemia and lipid, glucose and uric acid metabolism abnormalities (*4*). Therefore, it would be useful to determine the individual sensitivity to a TZD before prescribing it.

Regarding previous findings about gene polymorphisms that influence TZD-sensitivity, Turner *et al.* (*5*) reported that the β3-subunit of the G protein (*GNB3*) *C825T* polymorphism was related to the antihypertensive effect of a TZD in Caucasian and African-American subjects with essential hypertension (EHT). Glorioso *et al.* (*6*) also demonstrated that the α-adducin (*ADD1*) *Gly460Trp* polymorphism is the gene conferring susceptibility to the antihypertensive effect of TZDs in Italian hypertensives. This *ADD1 Gly460Trp* polymorphism was also suggested to confer susceptibility to salt-sensitivity in Caucasians and Asians with EHT (*7*).

Mutations of causative genes have recently been detected in several monogenic electrolyte disorders, such as mutations in the thiazide-sensitive Na-Cl cotransporter (*TSC*) gene for Gitelman syndrome (*8*, *9*), the WNK1 and 4 genes for Gordon syndrome (pseudohypoaldosteronism type II) (*10*) and the mineral corticoid receptor (*MLR*) for pseudohypoaldosteronism type I (PHA I) (*11*). TZDs are commonly effective for treating Gitelman syndrome and Gordon syndrome. We also focused on the Na^+/Ca^{2+} exchanger gene (*NCX1*), because its impairment was recently reported in mesangial cells from salt-sensitive hypertensive rats (*12*). TZDs are known to be effective for salt-sensitive hypertension. It is also known that the renin-angiotensin-aldosterone system (RAAS) and sympathetic nervous system (SNS) are activated in response to changes in circulating blood volume after TZD administration. Therefore, it is expected that gene polymorphisms related to the RAAS and SNS might be involved in the antihypertensive effect of TZDs. The present study investigated the gene polymorphism influencing the TZD-sensitivity by analyzing mainly single nucleotide polymorphisms (SNPs) of several water-electrolyte-related genes, including *GN3B*, *ADD1*, *TSC*, *MLR*, *NCX1*, *WNK1*, *WNK4* and RAAS- and SNS-related genes, to anticipate the effect of TZDs on BP in patients with hypertension.

Methods

Study Subjects

Peripheral blood samples for genetic analysis were collected with written informed consent from Japanese patients with EHT at an outpatient clinic of the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. This study protocol was approved by the Ethical Committee of the National Cardiovascular Center. Seventy-six patients, who had been newly prescribed TZDs as monotherapy or in addition to other antihypertensive agents, and whose blood-pressure data could be obtained from patients' records in 3 consecutive outpatient visits before and after starting TZDs, were retrospectively enrolled. BP was measured in the subjects after at least 10 min of rest in a sitting position. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) values were the means of three physician-obtained measurements. All subjects visited the outpatient clinic every month. The TZDs were a combination of indapamide (64.5%), trichlormethiazide (26.3%), mefruside (7.9%), and hydrochlorothiazide (1.3%). We defined patients who could achieve a BP reduction greater than 5 mmHg of mean blood pressure (MBP) after taking the TZDs as responders (R), and patients who could not achieve a BP reduction greater than 5 mmHg of MBP or showed increased BP after taking TZDs as non-responders (NR), according to the common evaluation criteria of antihypertensive drug effectiveness in Japan.

DNA Studies

Direct Sequencing for Detection of Polymorphisms in TSC, MLR, WNK1, WNK4, *and* NCX1

Genomic DNA was extracted using an NA-3000 nucleic acid isolation system (KURABO, Osaka, Japan) and stored at −80°C until use. We sequenced the 32–48 Japanese samples with written informed consent for genetic analysis. The methods used for the direct sequencing have been described previously (*13*). Briefly, all exons, part of the intron and an approximately 1,000-bp upstream region of exon 1, which would include the promoter regions of the *TSC*, *WNK1*, *WNK4* and *NCX* genes, were individually amplified by polymerase chain reaction and sequenced on an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, USA). In the *MLR* gene, exon 2 was sequenced. The polymorphisms were identified using the Sequencer software package (Gene Codes Corp., Ann Arbor, USA), followed by visual inspection.

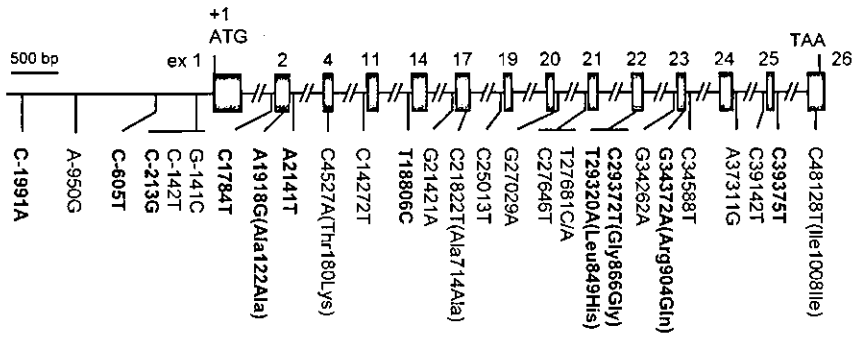

Fig. 1. *Location of genetic variants identified in* TSC. *Nucleotide numbers were counted from the initiation codon (ATG). Sequencing regions are indicated by the bars above the schematic gene structure. The initiation codon, ATG, and stop codon, TAA, are also shown. The single nucleotide polymorphisms presented in bold were genotyped in this study.*

Genotyping of Polymorphisms

The polymorphisms were genotyped using the TaqMan-polymelase chain reaction (PCR) system. Regarding genotyped SNPs, *C825T* of *GNB3* and *G29071T-Gly460Trp* of *ADD1* were selected according to previous studies (*5*, *6*, *14*). As the RAAS-related genes, *angiotensinogen* (*AGT*: *A−20C*, *G−6A*) (*15*, *16*), *ACE* (*G12568C* for *I/D*) (*17*), *angiotensin II type 1 receptor* (*AT1*: *A1166C*, *A−153G*) (*18*, *19*), and *aldosterone synthase* (*CYP11B2*: *C−344T*) (*20*) were tested. Furthermore, as the SNS-related genes, *adrenergic receptor β-1* (*ADRB1*: *A393G-Ser49Gly*, *G1413C-Arg389Gly*) (*21*), *β-2* (*ADRB2*: *C−47T*, *G2118A-Gly16Arg*, *G2151C-Glu27Gln*) (*22*, *23*), *β-3* (*ADRB3*:*T727C-Trp64Arg*) (*24*), *α-1a* (*ADRA1A*: *T44653C-Arg492Cys*) (*25*), *α-1b* (*ADRA1B*: *G834A*, *G1167A*) (*26*) and *α-2a* (*ADRA2A*: *A3023G*) (*27*) were tested for TZD sensitivity. Regarding *ACE*, we genotyped *G12568C* instead for the *I/D* polymorphism of 287 bp in intron 16 because *G12568C* showed almost complete linkage disequilibrium (LD) with the *I/D* polymorphism, as reported previously (*17*). For the directly sequenced genes, SNPs having a minor allele frequency of greater than 5% were selected for genotyping. As a result, 11 SNPs of *TSC* (Fig. 1), 2 SNPs of *MLR*, 7 SNPs of *WNK1*, 2 SNPs of *WNK4* and 7 SNPs of *NCX1* were genotyped. The sequences of the allele-specific probes and PCR primers used for the genotyping are shown in Table A1 in Appendix.

Statistical Analysis

Values are expressed as the means±SD. Hardy-Weinberg equilibrium was assessed by χ^2 analysis. The overall distribution of alleles was analyzed by χ^2 analysis. The distribution of genotypes between R and NR was analyzed by 2×2 contingency tables with a 2-sided Fisher exact probability test. The statistical significance was established at $p<0.05$. Comparison of BP reduction between allelic variants was performed by ANOVA followed by the Fisher protected least significant difference test using Stat-View version 5.0 (SAS Institute Inc., Cary, USA). LD and haplotype analyses were performed using the SNPAlyze statistical package version 2.1 (DYNACOM Co., Ltd., Mobara, Japan). The LD between SNPs was calculated by r^2. Tight LD was regarded as $r^2 \geq 0.5$. Haplotype estimation was performed by the expectation-maximization algorithm.

Results

Group Characteristics

Overall BP was significantly reduced after TZD administration (Fig. 2). Table 1 shows the group characteristics of R and NR. Forty-five patients who showed an MBP reduction of greater than 5 mmHg were defined as R, and 31 patients were defined as NR. Neither averaged age nor body mass index (BMI) showed a significant difference between R and NR. The BP before TZD administration was significantly higher in R than in NR. After TZD treatment, the averaged BP in R was remarkably decreased; however, the averaged BP in NR was slightly higher than that at pretreatment (Table 1). Control for deviation from Hardy-Weinberg equilibrium gave non-significant results in most SNPs examined in the present study, except *ADRA1B G1167A*. In the genotyping of *ADRA1B G1167A*, all of the study subjects were homozygous for the major allele, GG. This suggests that polymorphism of *ADRA1B G1167A* might not exist in the Japanese population.

Detection of Genetic Variants

We detected 52 SNPs of *TSC*, 7 SNPs of *MLR*, 35 SNPs of *WNK1*, 22 SNPs of *WNK4* and 15 SNPs of *NCX1* (Table A2 in Appendix). We confirmed some of the identified SNPs in the public database, dbSNPs (http://www.ncbi.nlm.nih.gov/SNP/). As shown in Table A2 in Appendix, some SNPs were very rare. Therefore, we chose SNPs that had a minor allele frequency of greater than 5% for genotyping by the TaqMan

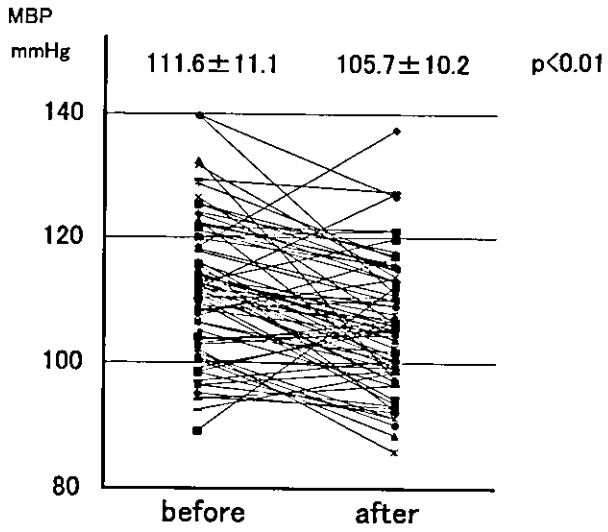

Fig. 2. *Mean blood pressure (MBP) before and after treatment with thiazide diuretics (TZDs) in all subjects. The average MBP in all subjects was significantly reduced by treatment with TZDs.*

method. Furthermore, some of these polymorphisms showed LD with other polymorphisms. Thus, we chose one SNP from among the polymorphisms with tight LD (*28*) ($r^2 \geq 0.5$) for genotyping the subjects. Therefore, genotyping by the TaqMan method was finally performed for 11 SNPs in *TSC*, 2 SNPs in *MLR*, 7 SNPs in *WNK1*, 2 SNPs in *WNK4* and 7 SNPs in *NCX1* (Table A1 in Appendix).

Association Study for the Effect of TZDs

No polymorphisms of the *MLR*, *WNK1*, *WNK4*, *NCX1*, and RAAS genes, and no polymorphisms of most of the SNS genes examined in the present study, including *C825T GNB3* and *Gly460Trp ADD1*, were significantly related to the effect of the TZDs, based on the comparison of each allele frequency between R and NR (Table 2). Two SNPs, *TSC C1784T* and *ADRB3 T727C*, showed a significant correlation with the effect of the TZDs (Table 3). The BP in patients with the homozygotes of the major allele of both *TSC C1784T* and *ADRB3 T727C* were significantly reduced by TZD treatment; however, the BP in those with the homozygotes of the minor allele and heterozygote (*TSC C1784T*: TT+CT; *ADRB3 T727C*: CC+CT) of both SNPs were not significantly changed after TZD treatment (Fig. 3). Furthermore, there was a much more significant difference in prevalence between the patients with the homozygotes of the minor alleles and heterozygote of these two SNPs, *TSC C1784T* and *ADRB3 T727C*, combined and those with the homozygotes of the major allele in R and NR than in *TSC C1784T* or *ADRB3 T727C* alone (odds ratio [OR]=5.09, $p=0.003$, confidence interval [CI]: 1.82–14.23).

Table 1. Comparison of Patients Characteristics between R and NR of TZD

	R (±SD)	NR (±SD)	*p* value
Number	45	31	
Age (years)	64.5±9.3	66.7±8.6	N.S.
Sex (male/female)	18/27	20/11	<0.05
BMI (kg/m^2)	24.7±3.2	24.7±3.2	N.S.
Pre SBP (mmHg)	157.9±14.3	142.9±15.0	<0.01
Pre DBP (mmHg)	95.0±10.7	86.5±10.1	<0.01
Pre MBP (mmHg)	115.9±9.7	105.3±9.9	<0.01
Pre HR (/min)	71.0±7.9	72.4±7.9	N.S.
Post SBP (mmHg)	138.8±15.0	147.2±17.4	<0.05
Post DBP (mmHg)	87.0±9.9	88.2±11.0	N.S.
Post MBP (mmHg)	104.3±9.3	107.9±11.2	N.S.
Post HR (/min)	72.4±9.3	72.8±9.0	N.S.
Monotherapy (%)	28.9	25.8	N.S.
Kind of TZD (%)			
Indapamide	66.7	61.3	N.S.
Trichlormethiazide	26.7	25.8	N.S.
Mefruside	6.7	9.7	N.S.
Hydrochlorothiazide	0.0	3.2	N.S.

R, responder; NR, non-responder; TZD, thiazide diuretics; BMI, body mass index; Pre SBP, systolic blood pressure at pretreatment; Pre DBP, diastolic blood pressure at pretreatment; Pre MBP, mean blood pressure at pretreatment; Pre HR, heart rate at pretreatment; Post SBP, systolic blood pressure at posttreatment; Post DBP, diastolic blood pressure at posttreatment; Post MBP, mean blood pressure at posttreatment; Post HR, heart rate at posttreatment; Monotherapy, prevalence of monotherapy by thiazide diuretics; Kind of TZD, prescribed kinds of TZD; N.S., not significant.

Haplotype Analysis

We measured the LD to understand the haplotype distribution of *TSC C1784T* in Japanese. There was a strong LD between the multiple SNPs within *TSC*. *TSC* was composed of three LD blocks, and the LD block containing *C1784T* consisted of two SNPs, *C−213G* and *C1784T*. The haplotype frequency was calculated for these two SNPs and the differences in haplotype distribution were compared between R and NR (Table 4). The results showed that the haplotype H2 with the 1784T allele tended to be different between the two groups, although not significantly so ($p=0.094$).

Discussion

The present study demonstrated that *TSC C1784T* and *ADRB3 T727C* were associated with the antihypertensive effect of TZDs in Japanese patients with EHT. A hypertensive patient with the minor homozygote or heterozygote of these two SNPs is predicted to be a non-responder to TZDs.

The TSC is present in the distal convoluted tubule, which

Table 2. Comparison of Allele Frequency between R and NR to TZD

Gene	Minor allele *vs.* common allele			
	SNP	Odds ratio	*p*	95% CI
ADD1	*Gly460Trp*	1.300	0.427	0.680–2.487
GNB3	*C825T*	1.620	0.146	0.844–3.110
TSC	*C−1991A*	1.483	0.586	0.356–6.167
	C−605T	1.483	0.586	0.356–6.167
	C−213G	1.483	0.586	0.356–6.167
	C1784T	3.816	0.013	1.253–11.627
	A1918G	0.594	0.235	0.251–1.410
	A2141T	2.378	0.102	0.821–6.886
	T18806C	0.825	0.566	0.428–1.591
	T29320A	1.459	0.790	0.090–23.770
	C29372T	1.143	0.718	0.554–2.359
	G34372A	—	0.147	—
	C39375T	0.681	0.311	0.323–1.436
MLR	*C−2G*	1.147	0.731	0.524–2.509
	G538A	0.685	0.418	0.274–1.716
WNK1	*G786A*	1.021	0.965	0.407–2.561
	C108560T	1.026	0.967	0.310–3.400
	A128177C	1.124	0.791	0.473–2.673
	C133634T	1.189	0.721	0.461–3.067
	G135642T	0.950	0.881	0.484–1.864
	C141114T	0.820	0.617	0.377–1.785
	C142763T	0.967	0.971	0.157–5.961
WNK4	*C14597T*	1.467	0.704	0.201–10.700
	C14717T	1.780	0.287	0.609–5.203
NCX1	*T−23690C*	0.849	0.721	0.346–2.084
	C−23449A	0.864	0.846	0.199–3.757
	T−23200C	0.651	0.553	0.156–2.711
	T−23181C	0.850	0.633	0.436–1.656
	A−22729C	0.914	0.861	0.334–2.505
	C−22144G	2.967	0.357	0.263–33.454
	G252581A	0.906	0.779	0.456–1.802
AGT	*A−20C*	1.265	0.540	0.596–2.687
	G−6A	0.758	0.527	0.320–1.793
ACE	*G12568C (I/D)*	0.768	0.443	0.392–1.508
AT1-R	*A1166C*	0.712	0.639	0.171–2.961
	A−153G	1.172	0.818	0.302–4.552
CYP11B2	*C−344T*	1.554	0.219	0.768–3.145
ADRB1	*G1413C*	1.724	0.228	0.707–4.204
	A393G	0.692	0.432	0.276–1.738
ADRB2	*C−47T*	1.098	0.869	0.361–3.338
	G2118A	1.531	0.203	0.793–2.956
	G2151C	1.228	0.744	0.358–4.217
ADRB3	*T727C*	4.591	0.003	1.541–13.680
ADRA1A	*T44653C*	0.630	0.412	0.207–1.913
ADRA1B	*G834A*	1.381	0.333	0.718–2.657
	G1167A	—	—	—
ADRA2A	*A3023G*	1.223	0.556	0.626–2.389

SNP, single nucleotide polymorphism; R, responder; NR, non-responder; TZD, thiazide diuretics; CI, confidence interval.

has been suggested to be the principal mediator of sodium and chloride reabsorption in this segment of the nephron. Simon *et al.* (*9*) demonstrated complete linkage of Gitleman syndrome to the genetic variants of *TSC*. The loss of function of TSC in patients with *TSC* gene variants could lead to low blood pressure, hypokalemic alkalosis, hypomagnesaemia and hypocalciuria (*29*). Melander *et al.* reported that gene polymorphism of TSC may influence EHT (*30*). It has recently been reported that TSC might interact with WNK4, which is one of the causative genes of Gordon syndrome (*31*, *32*). TZDs are generally effective in patients with Gordon syndrome. This would be due to the interaction between WNK and TSC. Four kinds of TZDs were used for the present study, and the main pharmacological mechanism of the antihypertensive effect common among those four TZDs was the blockage of TSC in the distal tubule. For these reasons, we expected that the gene polymorphisms of *TSC*, *WNK1* and *WNK4* might be related to the effect of TZDs. One SNP of *TSC* showed a significant association with the effect of the TZDs; however, there were no positive SNPs in either *WNK1* or *WNK4*.

TSC consists of 26 exons and is located on 16q13. We performed direct sequencing for *TSC*, including the promoter region, and detected 52 SNPs. We screened 11 SNPs that showed a minor allele frequency of greater than 5% for TZD sensitivity (Fig. 1). As a consequence, only *TSC C1784T* showed a significant correlation with the effect of TZDs. Since *TSC C1784T* is located in intron 1, as shown in Fig. 1, this SNP itself may not influence TSC function. Although we could not find functional polymorphisms linked with *C1784T*, there may be functional polymorphisms in much further upstream regions of the promoter or genes adjacent to *TSC*.

Administration of a TZD often induces activation of the RAAS and/or SNS as a result of circulating volume reduction. Thus, we investigated the participation of gene polymorphisms of the RAAS and SNS. The gene polymorphisms were selected from previous studies investigating the correlation between BP regulation and gene polymorphisms (*33*). Although Sciarrone *et al.* (*34*). reported that Caucasian hypertensive patients with the *I* allele of *ACE I/D* were more sensitive for hydrochrolthiazide than those with *DD*, no polymorphisms of the RAAS-related genes, including *ACE I/D*, showed a significant correlation with the effect of TZDs in the present study. Furthermore, neither *C825T* of *GNB3* (*5*) nor *Gly460Trp* of *ADD1* (*6*, *34*), which have previously been reported to influence the sensitivity to TZDs, showed a significant correlation with the effect of TZDs in the present study. It is suggested that the reason for the difference between the present findings and previous findings on the participation of the RAAS genes, *GNB3* and *ADD1*, in the effects of TZDs might be the ethnicity of the study subjects. Most studies (*5*, *34*) investigated the participation of the RAAS genes, *GNB3* and *ADD1*, in the effect of TZDs in Caucasians. In contrast, all subjects in the present study

Table 3. TZD- Sensitive Gene Polymorphisms of *TSC C1784T* and *ADRB3 T727C*

SNP	Sex	Genotype	R	NR	χ^2	p
TSC C1784T	M+F	CC	40	20	6.052	0.049
		CT	5	9		
		TT	0	1		
		CC	40	20	5.556	0.037
		TT+CT	5	10		
	Odds ratio=4.000, 95% CI=1.204–13.284					
		C allele	85	49	6.168	0.016
		T allele	5	11		
	Odds ratio=3.816, 95% CI=1.253–11.627					
ADRB3 T727C	M+F	CC	1	1	10.649	0.005
		CT	3	11		
		TT	40	18		
		TT	40	18	10.056	0.003
		CC+CT	4	12		
	Odds ratio=6.667, 95% CI=1.889–23.525					
		C allele	5	13	8.533	0.005
		T allele	83	47		
	Odds ratio=4.591, 95% CI=1.541–13.680					

TZD, thiazide diuretics; R, responder; NR, non-responder; SNP, single nucleotide polymorphism; M, male; F, female; CI, confidence interval.

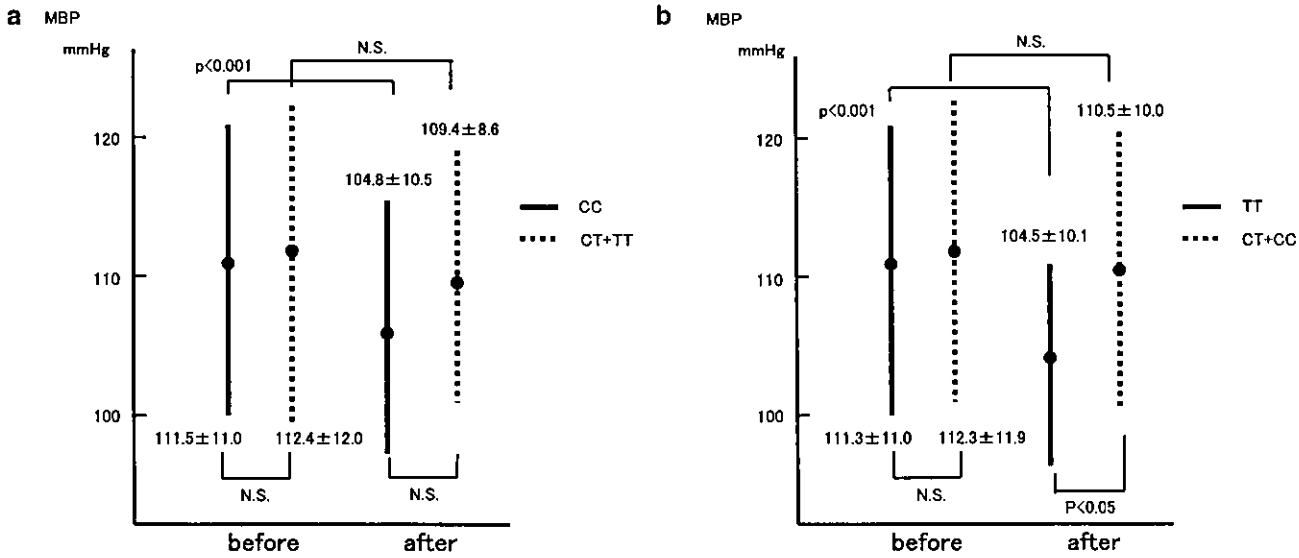

Fig. 3. *a: Comparison of the MBP changes before and after TZD therapy between homozygotes of the major allele CC and the minor homo- and heterozygote TT+CT of* TSC C1784T. *NS, not significant. b: Comparison of MBP changes before and after TZD administration between the homozygotes of the major allele TT and the minor homo- and heterozygote CC+CT of* ADRB3 T727C. *NS, not significant.*

were Japanese. The amount of salt intake in the Japanese population is generally greater than that in Caucasians (*35*). Thus, there is a possibility that Japanese may be more sensitive to TZDs than Caucasians.

The present study revealed a significant correlation between *T727C-Trp64Arg ADRB3* and the effect of TZDs. The β3 adrenergic receptors are mainly distributed in adipose tissues and involved in the regulation of lipolysis and thermogenesis. Regarding the *ADRB3* polymorphism *Trp64Arg*, it has been correlated with obesity (*36*), insulin resistance (*37*) and diabetes (*38*). In contrast, the relation between *Trp64Arg ADRB3* and hypertension is controversial (*24, 39, 40*). Re-

Table 4. Haplotype Distribution in R and NR in Two SNPs of *TSC*

Haplotype		R (n=90)	NR (n=60)	p value
H1	CC	81	47	0.622
H2	CT	5	9	0.094
H3	GC	4	2	>0.999
H4	GT	0	2	0.165

R, responder for thiazide diuretics: NR, non-responder for thiazide diuretics; SNPs, single nucleotide polymorphisms. Haplotypes were shown as combined alleles of genotyped 2 SNPs (*C−213G, C1784T*) of *TSC*.

garding the SNS activity, Shihara *et al.* (*41*) reported that subjects with the homo- and heterozygote of *64Arg ADRB3* had higher responses of the autonomic nerve activities after postural change than those with the wild-type gene. This suggests that *Trp64Arg ADRB3* may play an important role in the autonomic nervous system activities, including the activities of the SNS. To date, however, there has been no evidence that β3 adrenergic receptors exist and function physiologically on sympathetic nerves or the renal tubular system. Therefore, the mechanisms intervening between *ADRB3* gene polymorphism and the effectiveness of TZDs are unclear. Further investigations, including studies on the reflective activation of SNS and the worsening in insulin resistance after TZD administration, will be needed to clarify this interaction.

There is a question as to whether the contributions of *TSC C1784T* and *ADRB3 T727C* to the effects of TZDs are a TZD-specific finding. We tried to investigate the relationship between these 2 SNPs and the antihypertensive effects of an ACEI in 98 patients with EHT by the same study protocol; however, these 2 SNPs did not show any significant correlation to the effect of ACEI (unpublished data). Although investigation of other antihypertensive drugs is necessary, we speculate that the contribution of these 2 SNPs to the effect of TZDs is in fact a TZD-specific finding.

The study limitations include the retrospective design and the small sample size. The study subjects included not only patients receiving monotherapy with TZDs but also those receiving combined therapy with TZDs and other antihypertensive drugs. This issue is not considered to have much influence on the relationship between the 2 SNPs, *TSC C1784T-ADRB3 T727C*, and the effect of TZDs, because the prevalence of patients with monotherapy using TZD and the variation of the kinds of TZDs were not significantly different in between each allele of the two SNPs. However, a prospective and large-scale controlled study using TZDs is needed to confirm the importance of *TSC C1784T* and *ADRB3 T727C* on the antihypertensive effect of TZDs.

Furthermore, the BP level at pretreatment is considered an important factor in the effect of antihypertensive drugs. In the present study, BP before TZD administration was significantly higher in R than in NR. However, the BP level before TZD administration was not significantly different between TZD-sensitive and -insensitive genotypes in both *TSC C1784T* and *ADRB3 T727C-Trp64Arg*, as shown in Fig. 3. It might be possible that the BP response to TZDs was modified by the placebo effect. However, it is unlikely that *TSC C1784T* or *ADRB3 T727C* is involved in the placebo effect. A placebo-controlled prospective trial or ambulatory BP monitoring would help to confirm the significance of these SNPs in the BP-lowering effect of TZDs.

Finally, regarding the statistical approach, the Bonferroni method was not performed in this study even though multiple SNPs were investigated. The criterion for significance is $p<0.001$ (=0.05/48 SNPs) according to the Bonferroni method; however, *TSC C1784T* and *ADRB3 T727C* were associated with the antihypertensive effect of TZDs at $p=$ 0.016–0.049 and 0.003–0.005, respectively (Table 3). Although this might be considered a weak correlation for this kind of genetic research, we consider these two SNPs as prominent candidates relating to the effectiveness of TZDs, because both *TSC* and *ADRB3* were suggested to play an important role in the effectiveness of TZDs in patients with EHT, as we mentioned above.

In conclusion, *TSC C1784T* and *ADRB3 T727C-Trp64Arg*, may be gene polymorphisms susceptible to the antihypertensive effect of TZDs in patients with EHT. Thus, the prediction of BP reduction by TZDs may be possible by evaluating these two SNPs. Since the publication of the JNC 7, TZDs are becoming increasingly important as first-line drugs (*1*). The prediction of the TZD sensitivity of patients may lead to the realization of individualized therapy for hypertension based on genetic background.

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Appendix

The sequences of the allele-specific probes and PCR primers for the genotyping are shown in Table A1 and SNPs of *TSC*, *MLR*, *WNK1*, *WNK4* and *NCX1* are shown in Table A2.

References

1. Chobanian AV, Bakris GL, Black HR, *et al*: The seventh report of the Joint National Committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 Report. *JAMA* 2003; **289**: 2560–2572.
2. Guideline Subcommittee: 1999 World Health Organization-International Society of Hypertension guidelines for the management of hypertension. *J Hypertens* 1999; **17**:

Table A1. List of Genotyping Conditions for TaqMan PCR Method

Gene name	SNP	Primer	Final conc. (nmol/l)	Probe	Final conc. (nmol/l)	96-well annealing temp. and cycle no.	384-well annealing temp. and cycle no.
ADD1	*Gly460Trp*	CACACCTTAGTCTTCGACTTGGG	800	Fam-TTCTGCCCTTCCTC-MGB	200		58°C
	(G2907IT)	ACAAGATGGCTGAACTCTGGC	800	Vic-TTCTGCCATTCCTC-MGB	200		40
GNB3	*C825T*	CAGACCAGGAGCTGATCTGCTT	800	Fam-CATCACGTCCGTGGC-MGB	200		60°C
		TTGCAGTTGAAGTCGTCGTAGC	800	Vic-ATCACGTCTGTGGCCT-MGB	200		40
TSC	*C−1991A*	CCCTGACAGCTCAAATTTCCAC	800	Fam-CTGCCTCCCTGCAA-MGB	200		58°C
		CTTGTTACCAGAGGTGCCTAAGC	800	Vic-CTGCCTCACTGCAA-MGB	200		40
	C−605T	GCAGAAATGAAATCCACAAGCA	800	FAM-TTTGAAAATCCCTGTCCTG-MGB	228	62°C	58°C
		CATGCACCGATCATTAGATTGG	800	VIC-CTTTGAAAATTCCTGTCCTG-MGB	223	40	40
	C−213G	GGCAGAACACCATTTGATTGTG	800	FAM-CTGGCCCAAAGCCAGCCACTC-TAMRA	256	62°C	60°C
		GAAGAGCCACTCCAGGACTCA	800	VIC-CTGGCCCAAACCCAGCCACTC-TAMRA	282	35	40
	C1784T	CGCAGTGGTGCAGGTCAGT	800	Fam-CAGAGACGCCGTCC-MGB	200		58°C
		AGGTGTCTGCCTTCCTGCTG	800	Vic-TGCAGAGATGCCGTC-MGB	200		40
	A1918G	CTCACCATCACCCCCTTGAC	800	Fam-CTGGTGCCTGCCTCGCCC-TAMRA	200		60°C
		CAGCAGGAAGGCAGACACCT	800	Vic-TGGTGCCCGCCTCGCC-TAMRA	200		40
	A2141T	GCTTCAGTTTCCCCATCTGTACA	800	Fam-AATAGATTAAAGCCTGCCGG-MGB	200		58°C
		GGTGGCTTTTTAGGGAAACACA	800	Vic-AATAGATTAATGCCTGCCGG-MGB	200		40
	C4527A	GATGAACGTAGGTCGCATGGT	800	FAM-TGTCGGTCACGGTGA-MGB	336	60°C	58°C
		GATGGCTGAGATGGAGAGGC	800	VIC-TGTCGGTCAAGGTG-MGB	297	40	40
	T18806C	AGCAGCTCTGGCCTAGAAAGAG	800	FAM-TGGTGCCCTTGGCCCAGG-TAMRA	330	62°C	62°C
		ACGGAGATGATAGCCCCAAAC	800	VIC-CTGGTGCCCTCGGCCCAG-TAMRA	290	35	40
	T29320A	TCACATAGTGCTCTGTCCTGAGTG	800	FAM-TCCCTATCTCCTTGGC-MGB	242	62°C	60°C
		GATCTTGCATTTGCTCCACCTC	800	VIC-CCTATCACCTTGGCC-MGB	201	40	40
	C29372T	GCAAGAGGAGGTGGAGCAAAT	800	FAM-TTCGTAGGCGGCCAG-MGB	117	60°C	58°C
		CCCTCCACACTTACGCCTTTC	800	VIC-TCGTAGGTGGCCAGAT-MGB	254	40	40
	G34372A	GGGATTCCATGAAGTCCACATC	800	FAM-AACCCTCGGGCTGA-MGB	337	62°C	—
		CTGGAAGCCCCAAAACAGAAC	800	VIC-AGAACCCTCAGGCTG-MGB	329	40	—
	C39375T	GAAGCAGAAGGGCCAAAGTTC	800	FAM-ATAGCCCTGGCGATT-MGB	267	58°C	58°C
		GATGCCTGGGACACGTGAG	800	VIC-TAGCCCTGGTGATTC-MGB	84	40	40
MLR	*C−2G*	TTGTGGCTTAGCAAATGCAATT	800	Fam-TTTGTTAGCGATGGAGAC-MGB	602	62°C	
		CAGGGAGACTGTGGTAGCCTTT	800	Vic-ATTTGTTAGGGATGGAGAC-MGB	224	40	
	G538A	GGGCTTTTCTCATGACACATGATA	800	Fam-CTTTTAACAATGGCGCGC-MGB	189		60°C
		CGCCCTTGAGATCATTTATGTCT	800	Vic-TTTAACAACGGCGCGCA-MGB	361		40
NCX1	*T−23690C*	CTCTCCCCACAGGTCATTCTG	800	Fam-ATTTAACTTATAGCAAGGAA-MGB	200		58°C
		GCAGGAATCGTTCTTGCCTAA	800	Vic-TTAACTTACAGCAAGGAA-MGB	200		40
	C−23449A	GAATCTGCAATCCCCATGTGAT	800	Fam-CTCACATTCATGTTTGAG-MGB	200		56°C
		AGAACCACTGCTCTAGGCCAAT	800	Vic-ACTCACATTAATGTTTGAGG-MGB	200		40
	T−23200C	TTCTGAGGTGCAAGGAGGGTT	800	Fam-CCCCCTTTTTGTTGC-MGB	100		56°C
		GGCAGTCACCACGACTGATAGA	800	Vic-CCCCCTCTTTGTTG-MGB	100		40
	T−23181C	GGCAGTCACCACGACTGATAGA	800	Fam-TCCAGGAACCTCAGTTT-MGB	200		56°C
		AGGCTATTTCTTCCATTCCGC	800	Vic-CCAGGAACCTCGGTTT-MGB	200		40
	A−22729C	GCCTGGTGCAGTGTTCCTTTA	800	Fam-ATTATGAGGAAAGTGATTTA-MGB	200		58°C
		GCCCTTTCCAAGAGAAGCATTA	800	Vic-TATGAGGACAGTGATTTA-MGB	200		40
	C−22144G	AAAAGAAAAGTTGCAGCGCCT	800	Fam-CCACAACGCACTGC-MGB	200		56°C
		TTTTTCGATTTCCTGCCGG	800	Vic-CACAAGGCACTGCG-MGB	100		40
	G252581A	AAACAAAGACATACCAGCGAGAAA	800	Fam-CTCTCTCCGTGTCTC-MGB	200		58°C
		AAATTGCTAAAGCTTCAAAGGCA	800	Vic-TCTCTCCATGTCTCC-MGB	200		40
WNK1	*G786A*	GAACTGCAGGTAAAGCCCCAC	800	Fam-TTTGACGGTCCTTTG-MGB	200		58°C
		GAACTCGATCAACTGGCTTCG	800	Vic-TTTATTTGACAGTCCTTTG-MGB	200		40
	C108560T	CTGATGGGACGGTTGACAGTG	800	Fam-TCTTCACAGAATCTCGA-MGB	200		58°C

Table A1. (Continued)

Gene name	SNP	Primer	Final conc. (nmol/l)	Probe	Final conc. (nmol/l)	96-well annealing temp. and cycle no.	384-well annealing temp. and cycle no.
		CCTGTTCATGTTGGGAACCATA	800	Vic-TCTTCATAGAATCTCG-MGB	200		40
	A128177C	GTTGCTCCTGCAGAGCCAGT	800	Fam-AGTAGCACAGACCCAA-MGB	200		58 °C
		TCTACAGAGGAAGCCAAAGTGGT	800	Vic-AGTAGCACAGCCCCA-MGB	200		40
	C133634T	TTGATTTGCTCTTCAGTACGCAG	800	Fam-AGCGTCTCACGGACT-MGB	200		58 °C
		GCACCTACAGACAACAAAGGGAA	800	Vic-AGCGTCTCATGGACT-MGB	200		40
	G135642T	AAAACTTACACCAACCGCAGAAG	800	Fam-CTGTGATCATCTCTG-MGB	200		58 °C
		ATTCAGTCCCAGCAACCTCTAGA	800	Vic-ACTGTGATAATCTCTG-MGB	200		40
	C141114T	TGGGACGATTTCAGGTAAGACAG	800	Fam-ATTCCTTCCTTTGGAGGA-MGB	200		58 °C
		TTGTGTCCCAAATAGGTAGGCA	800	Vic-ATTCCTTCTTTTGGAGGAG-MGB	200		40
	C142763T	ACGACCCACTTTGTTTGCTGTA	800	Fam-CTGAAAACGTCCAACCT-MGB	200		58 °C
		GTCAGACACTGGGCAGCCTAC	800	Vic-CCTGAAAACATCCAACCT-MGB	200		40
WNK4	*C14597T*	CTGGCTGTGATGACTGTGGC	800	Fam-TCCCCTCCCTAGCCT-MGB	200		58 °C
		TGAAGGGCTTTCCTGGCC	800	Vic-TCCCCTCTCTAGCCTG-MGB	200		40
	C14717T	CACAGCTGAGGTGGAGAGTGAG	800	Fam-CTCCACTCTGCACTC-MGB	200		58 °C
		GGAGGTGGTGAGGCCTAGAAA	800	Vic-ACTCCATTCTGCACTC-MGB	200		40
AGT	*A(−20)C**	CTTCTGGCATCTGTCCTTCTGG	250	Direct sequence			64 °C
		CTGGTCTTATGAGAGGGGAGAGG	250				35
	*G(−6)A**	Same as *A(−20)C*		Direct sequence			
ACE	*G12568C*	AGCAGAGGTGAGCTAAGGGCT	667	Fam-CTCAAGGCATTCAA-MGB	200		58 °C
	(I/D)	GGCCATCACATTCGTCAGATCT	667	Vic-CTCAAGCCATTCAA-MGB	200		40
AT1	*A(−153)G*	AACGCTGATCTGATAGTTGACACG	800	Fam-CCGTCAATATCCCGAG-MGB	200		60 °C
		CTCTGTTTTGCATTCCCTCCTC	800	Vic-CCGTCAGTATCCCGA-MGB	200		40
	A1166C	AGAGAACATTCCTCTGCAGCACT	800	Fam-CAAATGAGCATTAGCT-MGB	200		60 °C
		CGGTTCAGTCCACATAATGCAT	800	Vic-CAAATGAGCCTTAGCT-MGB	200		40
CYP11B2	*C(−344)T*	TGGACATTTTCTGCAGTTTTTGA	800	Fam-ATCCAAGGCTCCCTCT-MGB	100		56 °C
		TCCTTTCTCCAGGGCTGAGA	800	Vic-CAAGGCCCCCTCT-MGB	100		40
ADRB1	*G1413C*	TTCTTCAACTGGCTGGGCTAC	800	Fam-CCTTCCAGGGACTGC-MGB	200		58 °C
		GTCTCCGTGGGTCGCGT	800	Vic-CTTCCAGCGACTGCT-MGB	200		40
	A393G	CCGGTAACCTGTCGTCGG	800	Fam-CAGCGAAAGCCCCGA-MGB	200		58 °C
		GATCACCAGCACATTGCCC	800	Vic-AGCGAAGGCCCCGAG-MGB	100		40
ADRB2	*C(−47)T*	CATTGGGTGCCAGCAAGAA	800	Fam-CGCCTCAGCGGGCGGA-TAMRA	100		56 °C
		GAATGAGGCTTCCAGGCGT	800	Vic-CGCCTCAGCAGGCGGACC-TAMRA	100		40
	G2118A	CGCTGAATGAGGCTTCCAG	800	Fam-ACCCAATGGAAGCC-MGB	100		58 °C
		CTGCGTGACGTCGTGGTC	800	Vic-ACCCAATAGAAGCCA-MGB	100		40
	G2151C	CCAGGACGATGAGAGACATGAC	800	Fam-TCCCTTTCCTGCGTGA-MGB	200		58 °C
		CCTTCTTGCTGGCACCCA	800	Vic-TCCCTTTGCTGCGTG-MGB	200		40
ADRB3	*T727C*	CACGTTGGTCATGGTCTGGA	800	Fam-CGGAGTCCAGGCGA-MGB	200		58 °C
		GAGGCAACCTGCTGGTCATC	800	Vic-TCGGAGTCCGGGCG-MGB	200		40
ADRA1A	*T44653C*	TCCAGCCAAGAGTTCAAAAAGG	800	Fam-CAGTGTCTCTGCAGAA-MGB	100		56 °C
		CCAGGGCATGTTTGGAAGACT	800	AGTGTCTCCGCAGAA-MGB	200		40
ADRA1B	*G834A*	CGCACTCCTTGTCATCGTTG	800	Fam-TCCTTCCACCCAAGGA-MGB	200		58 °C
		GTCTTGTCCACCGTCATCTCC	800	Vic-CTCCTTCCATCCAAGGA-MGB	200		40
	G1167A	CAAGATGAACATACCGACCACAA	800	Fam-CCCAACGTCTTAGCT-MGB	200		60 °C
		CAACCCCAGGAGTTCCATAGC	800	Vic-CCCAACGTTTTAGCT-MGB	200		40
ADRA2A	*A3023G*	TCCCCTTCCATTCCCAACTC	800	Fam-TCTCTTTTTAAAGAAAAAT-MGB	200		56 °C
		TTCAACATCAAAACCAAGGCC	800	Vic-TCTTTTTGAAGAAAAAT-MGB	100		40

* The genotyping for *AGT A(−20)C* and *G(−6)* polymorphisms was performed by the direct sequence method. A pair of the PCR primers was 5′-CTTCTGGCATCTGTCCTTCTGG-3′ and 5′-CTGGTCTTATGAGAGGGGAGAGG-3′.

Table A2. List of 130 Polymorphisms and Their Allele Frequency in *TSC*, *MLR*, *WNK1*, *WNK4* and *NCX1* Genes Identified by the Direct Sequence

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
TSC	*C−1991A*		promoter	38	0	10	48	0.792	0.208	caccactgcctc[c/a]ctgcaatggctt	
	A−950G		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		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]gtacccctgct	
	−544delT		promoter	47	1	0	48	0.990	0.010	tcacgtacccccc[t/-]gcttgctcaatc	
	C−213G		promoter	35	8	0	43	0.907	0.093	gggagtggctgg[c/g]tttgggccagcc	
	C−142T		promoter	1	20	22	43	0.256	0.744	gtgttctgcctc[c/t]ggccctgtccgg	
	G−141C		promoter	28	15	0	43	0.826	0.174	tgttctgcctcc[g/c]gccctgtccggg	
	C1784T		intron1	30	17	1	48	0.802	0.198	tggatgcagaga[c/t]gccgtccctagc	
	A1918G	Ala122Ala	exon2	31	17	0	48	0.823	0.177	ggagggcgaggc[a/g]ggcaccagcagc	rs2304479
	A2141T		intron2	0	8	40	48	0.083	0.917	acaatagattaa[a/t]gcctgccgggga	rs2304480
	G2971A		intron2	47	1	0	48	0.990	0.010	tagggcctaggt[g/a]ctcgataccctg	
	C4527A	Thr180Lys	exon4	43	2	0	45	0.978	0.022	tgctgtcggtca[c/a]ggtgacctccat	
	C7479T	Phe341Phe	exon8	38	2	0	40	0.975	0.025	tggcaccttctt[c/t]ggaatgttctcc	
	C14272T		intron10	26	18	3	47	0.745	0.255	ctggctcagccc[c/t]caccgtggagtc	rs3816119
	G14277A		intron10	46	1	0	47	0.989	0.011	tcagccccacc[g/a]tggagtccctga	
	C14363A	Ala464Ala	exon11	45	2	0	47	0.979	0.021	catcttcggggc[c/a]accctctcctct	
	C14366T	Thr465Thr	exon11	46	1	0	47	0.989	0.011	cttcggggccac[c/t]ctctcctctgcc	rs5801
	G17337A		intron13	44	1	0	45	0.989	0.011	ggggtgggagtg[g/a]gaggcatgggtg	
	T18806C[b]		intron13	6	24	18	48	0.375	0.625	gactggtgccct[t/c]ggcccagggtgg	rs2304483
	C18850T	Ala569Val	exon14	46	2	0	48	0.979	0.021	acaacaagtggg[c/t]ggcgctgtttgg	
	T20072C	Leu623Pro	exon15	46	1	0	47	0.989	0.011	gctcctacaacc[t/c]ggccctcagcta	
	G20088A	Ser628Ser	exon15	46	1	0	47	0.989	0.011	cctcagctactc[g/a]gtgggcctcaat	
	C20201G		intron15	46	1	0	47	0.989	0.011	gagtttccaagc[c/g]tagacctgtcac	
	G21421A		intron16	20	24	3	47	0.681	0.319	atgggggcccaa[g/a]gggatgcggagc	
	C21500T		intron16	42	2	0	44	0.977	0.023	ccctcttgctgg[c/t]ttctcccccagc	
	C21566G		intron16	43	1	0	44	0.989	0.011	cactttctcccc[c/g]actccttgtgtt	
	A21586G		intron16	43	1	0	44	0.989	0.011	gtgttttcccct[a/g]tctgggcaaaag	
	C21822T	Ala714Ala	exon17	21	21	3	45	0.700	0.300	ggatgtcattgc[c/t]gaggacctccgc	
	C22682T		intron17	46	1	0	47	0.989	0.011	tcacccctatcc[c/t]ctggcaggccgc	
	C25013T[c]		intron18	23	22	3	48	0.708	0.292	ctgggggagaag[c/t]tggacctcacct	rs3764264
	G27029A		intron20	18	25	4	47	0.649	0.351	ttttcttgtgac[g/a]gtggtgcctgag	
	C27646T[b]		intron20	6	26	15	47	0.404	0.596	aaggggcgttgg[c/t]ggggccctgggc	rs2278490
	T27681C[b*]		intron20	5	23	18	47	0.351	0.628	tggatgcgcggc[t/c]gctggctctgct	rs2278489
	*A27681C**			0	1	—		0.011	—	tggatgcgcggc[a/c]gctggctctgct	
	*T27681A**			—	0	—		—	—	tggatgcgcggc[t/a]gctggctctgct	
	T29320A	Leu849His	exon22	367	5	0	372	0.993	0.007	tcattccctatc[t/a]ccttggccgcaa	
	C29372T[c]	Gly866Gly	exon22	23	22	3	48	0.708	0.292	tgtgttcgtagg[c/t]ggccagattaac	rs5804
	G34262A		intron22	44	1	3	48	0.927	0.073	tctcaagaaaaa[g/a]taataacaataa	
	G34372A[d]	Arg904Gln	exon23	45	3	0	48	0.969	0.031	accagaaccctc[g/a]ggctgagcagta	
	C34588T		intron23	41	3	4	48	0.885	0.115	cacagggcaagg[c/t]ggctgcagcccc	
	T37125C		intron23	46	1	0	47	0.989	0.011	cctcaacccact[t/c]tctcgtccccag	
	C37210T	Asn931Asn	exon24	46	1	0	47	0.989	0.011	ggccactgtcaa[c/t]gagatgcggcgg	
	A37311G[e]		intron24	23	21	3	47	0.713	0.287	acgcgacacatc[a/g]ctgggtcaggga	rs2289117
	G39097A		intron24	29	1	0	30	0.983	0.017	gaggccatagac[g/a]tggtgaaggatt	
	C39119T		intron24	29	1	0	30	0.983	0.017	attgagtgacct[c/t]gatgatatggga	
	C39142T		intron24	40	7	0	47	0.926	0.074	gaagtgaccact[c/t]ggctttctcccg	rs3816118
	G39143A[d]		intron24	44	3	0	47	0.968	0.032	aagtgaccactc[g/a]gctttctcccgc	rs2289116
	C39203T	Ser967Phe	exon25	46	1	0	47	0.989	0.011	tgctggattact[c/t]ccgagacgctgc	

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	acatcccctgtcc[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]ccgccccctgggg	
	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]acattccttta	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	tttcttttcccct[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	atcagtttttttt[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]ttggaggagtt	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	gagggggagaga[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]aactccttgtc	
	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	cacctccccttt[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]atgtttgaggag	
	T−23200C[m]		intron1d	0	9	86	95	0.047	0.953	attccgcccccc[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]tgtctccctact	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.

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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

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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[9]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

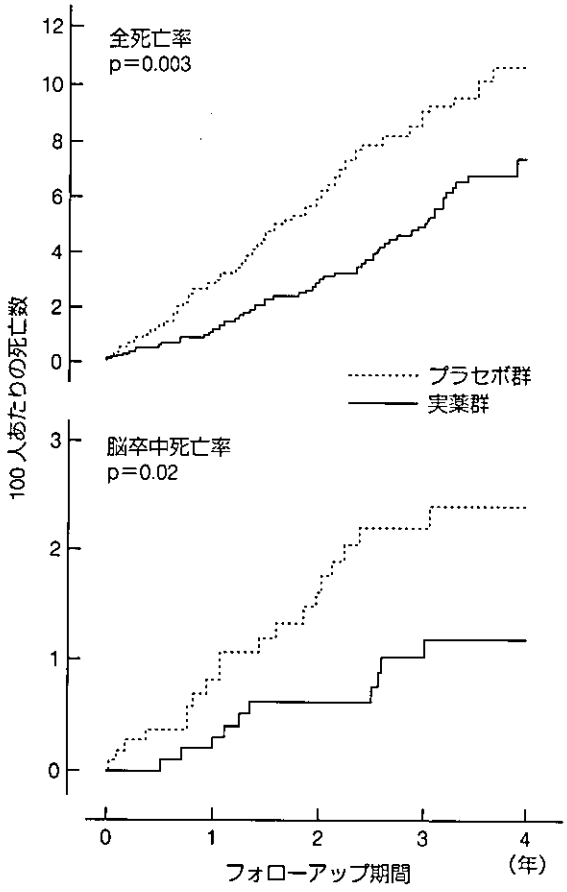

図 1. Syst-China 研究における実薬群とプラセボ群の全死亡率および脳卒中死亡率
(Liu L *et al*, 1998[9]より引用)

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

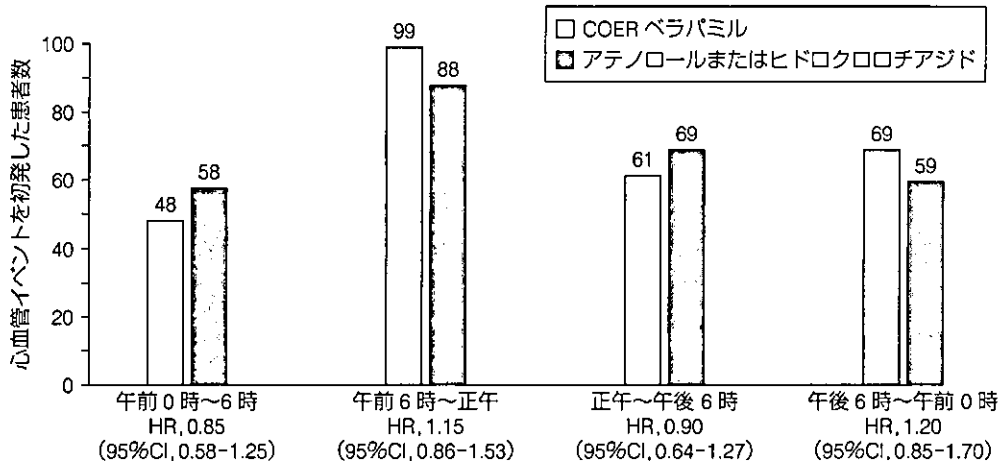

図 2. CONVINCE 試験における COER ベラパミル群とアテノロールまたはヒドロクロロチアジド群の時間別の心血管イベント
(Black HR *et al*, 2003[10]より引用)

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ヵ月後	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．

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Single Nucleotide Polymorphisms Analysis of Hypertension Relating to the Effect of Antihypertensive Drugs

- Millennium Genome Project at NCVC -

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A short title: SNPs analysis in essential hypertension

Abstract

The Millennium Genome Project for clarifying genes involved in hypertension is in progress at the National Cardiovascular Center (NCVC). We are performing investigations mostly on single nucleotide polymorphisms (SNPs) of candidate genes related to blood pressure elevation, hypertensive cardiovaseular complications and antihypertensive drug effects in patients with essential hypertension. In this paper, we describe an overview of genetic analysis in hypertension, and touch on our current findings on gene polymorphisms responsible for the effect of the antihypertensive drugs.

To detect the gene polymorphism sensitive to the effect of thiazide diuretics (TZD), 76 outpatients with essential hypertension were retrospectively categorized as responders (R) or non-responders (NR). A patient whose mean blood pressure was lowered by over 5 mmHg after TZD treatment was defined as a responder. Candidate genes related to water-electrolyte metabolism were examined in this study. Genotyping using the TaqMan PCR method for gene polymorphisms with a minor allele frequency over 5% in *thiazide- sensitive Na-Cl cotransporter* (*TSC* : 12 SNPs detected by direct sequences) and an other 5 genes and 18 polymorphisms was performed. The comparison of polymorphisms prevalence between R and NR showed a significant difference only in