Table 1. Comparison of Characteristics of Responders and Nonresponders to L-Type CCBs

	R=ΔSBP>20 mmHg			R=ΔDBP>10 mmHg		
	Responder (±SD)	Nonresponder (±SD)	P value	Responder (±SD)	Nonresponder (±SD)	P value
n	48	113		56	105	
Age (years)	62.7±10.8	66.7±9.0	0.016	63.9±11.3	66.4±8.6	0.124
Sex (M/F)	23/25	62/51	0.419	34/22	51/54	0.140
BMI (kg/m^2)	21.1±7.9	21.4±8.6	0.849	22.5±7.0	20.7±9.0	0.201
Pre-SBP (mmHg)	169.7±18.8	151.2±18.5	<0.001	161.8±23.8	154.0±17.8	0.020
Pre-DBP (mmHg)	102.9±11.5	93.6±10.0	<0.001	102.4±12.1	93.2±9.4	<0.001
Pre-MBP (mmHg)	125.2±12.2	112.8±9.2	<0.001	122.2±13.0	113.4±9.6	<0.001
Pre-HR (beats/min)	68.6±9.3	69.6±10.9	0.585	68.6±9.3	69.6±10.9	0.585
Post-SBP (mmHg)	137.6±14.2	146.3±15.1	<0.001	137.7±14.2	146.9±15.0	<0.001
Post-DBP (mmHg)	86.4±9.3	88.4±9.9	0.243	84.4±10.4	89.6±8.9	0.001
Post-MBP (mmHg)	103.5±9.3	107.7±9.7	0.012	102.2±9.3	108.7±9.2	<0.001
Post-HR (beats/min)	72.6±9.8	71.8±12.8	0.708	72.6±9.8	71.8±12.8	0.708
Monotherapy (%)	37.5	21.2	0.035	30.4	23.8	0.371
Type of CCB (%)						
Amlodipine	39.6	44.2	0.584	42.9	42.9	1.000
Nifedipine	22.9	18.6	0.533	23.2	18.1	0.442
Nicardipine	10.4	11.5	0.840	5.4	14.3	0.071
Manidipine	8.3	9.7	0.778	10.7	8.6	0.659
Nilvadipine	6.3	8	0.700	8.9	6.7	0.607
Benidipine	2.1	2.7	0.829	1.8	2.9	0.669
Nitrendipine	4.2	1.8	0.392	1.8	2.9	0.669
Bamidipine	0.0	1.8	0.232	0.0	1.9	0.189
Cilnidipine	2.1	0.9	0.548	1.8	0.9	0.657
Efonidipine	2.1	0.0	0.119	1.8	0.0	0.145

Responder defined as SBP reduction (ΔSBP)>20 mmHg or DBP reduction (ΔDBP)>10 mmHg, respectively, after taking L-type CCB.

CCBs, calcium-channel blockers; R, response; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; Pre-SBP, SBP before treatment; Pre-DBP, DBP before treatment; Pre-MBP, mean blood pressure before treatment; Pre-HR, heart rate before treatment; Post-SBP, SBP after treatment; Post-DBP, DBP after treatment; Post MBP, mean blood pressure after treatment; Post-HR, heart rate after treatment; Monotherapy, prevalence of monotherapy.

smooth muscle.[14] L-type calcium channels are formed by 1 of 4 principle pore-forming α1 subunits [α1S (Cav1.1), α1C (Cav1.2), α1D (Cav1.3), and α1F (Cav1.4)], which are encoded by different individual genes, in association with several auxiliary subunits.[15] Expression of α1S and of α1F is restricted to skeletal muscle and retina, respectively, but α1C and α1D are widely expressed in neuronal and (neuro)endocrine cells and in electrically excitable cells in the cardiovascular system, including cardiac muscle and vascular smooth muscle.[16] In most cases, both channel types are found in the same cells, with α1C usually being the predominant isoform.[17] Although previous studies have shown that the effects of dCCBs on the contractility of ventricular muscle and aortic smooth muscle are exclusively mediated by α1C (not by α1D),[18] and that α1D might control physiological processes, such as diastolic depolarization in sinoatrial node cells and neurotransmitter release and neuronal excitability,[19] the physiological effects of these subunits are largely unknown. Considering their expression patterns, the central role of α1C on the contractility of heart muscle and of vascular smooth muscle, and the important role of the neuroendocrine system in the pathophysiology of HT,[20] genes encoding α1C (*CACNA1C*) or α1D (*CACNA1D*) might be candidates for influencing the antihypertensive effects of L-type dCCBs.

The aim of the present study was to evaluate *CACNA1C* and *CACNA1D*, which encode L-type calcium-channel subunits α1C and α1D, respectively, in relation to the responsiveness of patients with EHT to treatment with L-type dCCBs. We focused on evaluating the effects of the α1C subunit. First, we screened for possible genetic polymorphisms in the promoter region, all exon regions, and a small part of the intron regions of *CACNA1C* in 48 patients with HT. Next, we performed genotyping of the missense mutations and representative common polymorphisms of *CACNA1C* found with direct sequencing or common single nucleotide polymorphisms (SNPs) of *CACNA1D* chosen from a public database in 161 patients with EHT who were treated with L-type dCCBs. Finally, we examined the association of these genetic polymorphisms with the responsiveness of patients with EHT to treatment with L-type dCCBs.

Methods

Study Subjects

Peripheral blood samples for genetic analysis were collected after written informed consent was given by Japanese patients with EHT at an outpatient clinic of the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. The study protocol was approved by the Ethics Committee of the National Cardiovascular Center. A total of 161 patients (85 men, 76 women), for whom L-type dCCBs had been newly prescribed as monotherapy or in addition to other antihypertensive agents and for whom BP data could be obtained from records of 3 consecutive outpatient visits before and after the start of treatment with L-type dCCBs, were retrospectively enrolled. BP was measured in the subjects after they had rested while seated for at least 10 min. Systolic BP (SBP) and diastolic BP (DBP) values were the means of 3 physician-obtained measurements. All subjects visited the outpatient clinic every month. The L-type dCCBs prescribed were amlodipine (43.5%), nifedipine (19.9%), nicardipine (11.8%), manidipine (9.3%), nilvadipine (7.5%), benidipine (2.5%),

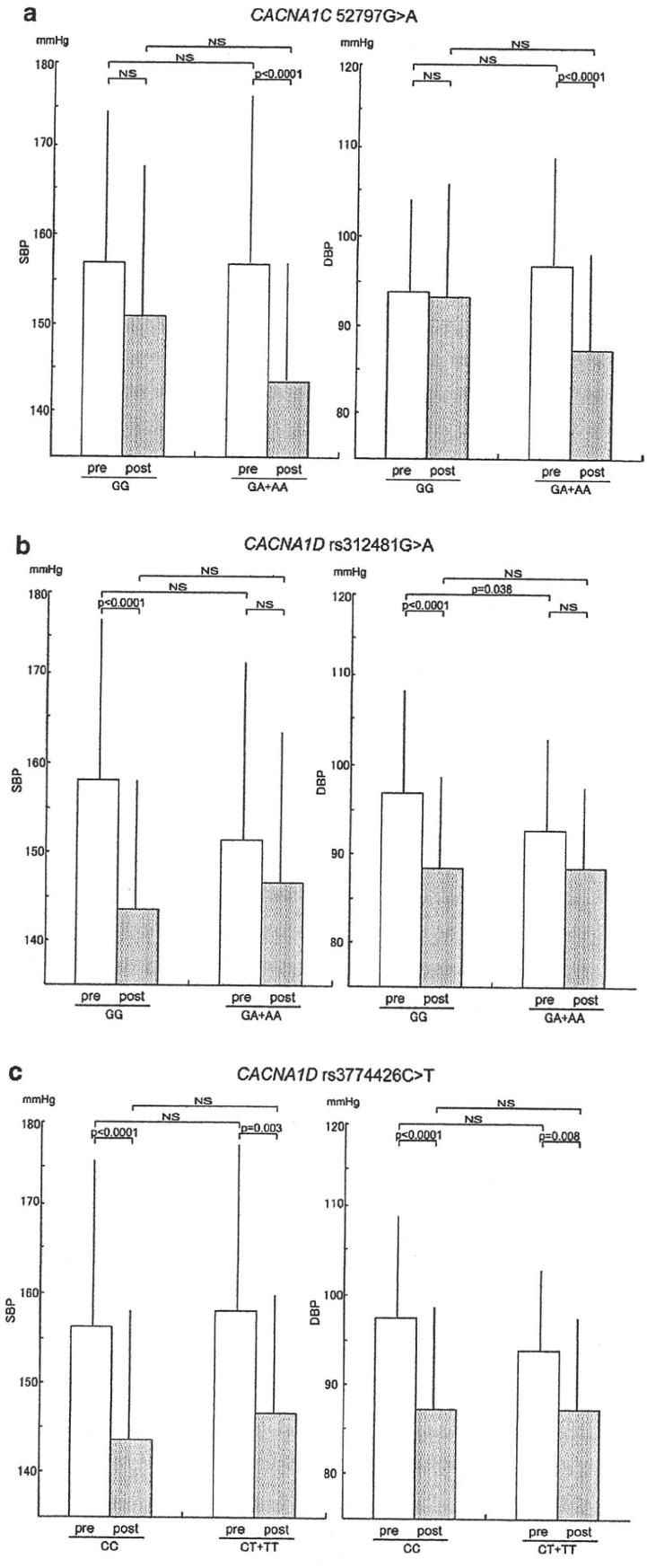

Figure. (a) Comparison of blood pressure (BP) between pre- and post-administration of CCBs in each genotype of dominant model in patients with *CACNA1C* 527974G>A. (b) Comparison of BP between pre- and post-administration of CCBs in each genotype of recessive model in patients with *CACNA1D* rs312481G>A. (c) Comparison of BP between pre- and post-administration of CCBs in each genotype of recessive model in patients with *CACNA1D* rs3774426C>T. CCBs, calcium-channel blockers; SBP, systolic BP; DBP, diastolic BP.

Table 2. Characteristics of Genetic Polymorphisms Identified by Direct Sequencing or Genotyping With TaqMan PCR

Gene, location	SNPs	LD	aa. Info	Region	Allele 1	Hetero	Allele 2	Total	Allele 1 freq.	Allele 2 freq.	TaqMan
*CACNA1C**	395458G>A		A174A	Exon 4	43	5	0	48	0.948	0.052	
12p 13.3	395570G>A	a		Intron 4	29 (102)	17 (48)	2 (11)	48 (161)	0.781 (0.783)	0.219 (0.217)	OK
	395572T>C	a		Intron 4	29	17	2	48	0.781	0.219	
	439886C>A			Intron 7	45	3	0	48	0.969	0.031	
	459184G>A	b		Intron 8	35 (137)	13 (22)	0 (1)	48 (160)	0.865 (0.919)	0.135 (0.081)	OK
	496317delG	c		Intron 9	30	14	0	44	0.841	0.159	
	496354G>T	c		Intron 9	30 (101)	18 (55)	0 (5)	48 (161)	0.813 (0.798)	0.187 (0.202)	OK
	513074G>A	b, c		Intron 12	32	13	0	45	0.856	0.144	
	513955C>T	d		Intron 12	0	2	46	48	0.021	0.979	
	527974 G>A	c, e		Intron 13	4 (8)	20 (62)	24 (91)	48 (161)	0.292 (0.242)	0.708 (0.758)	OK
	529458T>G	e, f		Intron 15	1	22	23	46	0.261	0.739	
	530778G>C			Intron 15	47	1	0	48	0.990	0.010	
	531126A>G			Intron 16	47	1	0	48	0.990	0.010	
	531910C>T	f	D812D	Exon 17	18	24	3	45	0.667	0.333	
	539757G>A		A879A	Exon 19	47	1	0	48	0.990	0.010	
	542532G>T	g		Intron 20	47	1	0	48	0.990	0.010	
	551409T>C	h		Intron 22	47	1	0	48	0.990	0.010	
	552959A>G	d		Intron 24	46	2	0	48	0.979	0.021	
	554886G>A			Intron 26	47	1	0	48	0.990	0.010	
	557206C>T	d		Intron 28	46	2	0	48	0.979	0.021	
	557231C>T			Intron 28	47	1	0	48	0.990	0.010	
	558260T>C	f		Intron 28	14	27	6	47	0.585	0.415	
	558409 C>T	f	F1262F	Exon 29	14 (58)	27 (79)	6 (24)	47 (161)	0.585 (0.606)	0.415 (0.394)	OK
	594891C>G			Intron 30	45	3	0	48	0.969	0.031	
	595028 T>C	i		Intron 31	8 (9)	18 (60)	22 (92)	48 (161)	0.354 (0.242)	0.646 (0.758)	OK
	595041T>C	i		Intron 31	8	18	22	48	0.354	0.646	
	595054C>T	i		Intron 31	8	18	22	48	0.354	0.646	
	597980 G>A	j		Intron 31	4 (14)	17 (39)	23 (108)	44 (161)	0.284 (0.208)	0.716 (0.792)	OK
	598239delA	j		Intron 32	4	17	23	44	0.284	0.716	
	615494delT	g		Intron 38	47	1	0	48	0.990	0.010	
	615546–615547insC	k		Intron 38	32	15	0	47	0.840	0.160	
	624139G>A	h		Intron 40	43	1	0	44	0.989	0.011	
	624330 C>T	k		Intron 41	33 (105)	15 (46)	0 (10)	48 (161)	0.844 (0.795)	0.156 (0.205)	OK
	626151G>A		T1787T	Exon 43	8	17	16	41	0.402	0.598	
	632652 G>A		R1910Q	Exon 45	45 (159)	3 (2)	0 (0)	48 (161)	0.969 (0.994)	0.031 (0.006)	OK
	635110 G>A		G2004S	Exon 46	35 (160)	1 (0)	0 (0)	36 (160)	0.986 (1.000)	0.014 (0.000)	OK
	637259C>T	j, l		Intron 46	28	17	3	48	0.760	0.240	
	638741–638742insT	l		3'-UTR	28	17	3	31	0.875	0.125	Failed
*CACNA1D***	rs3774414 C>T			Intron 2	64	78	18	160	0.644	0.356	OK
3p 14.3	rs219847 G>A			Intron 2	40	82	38	160	0.506	0.494	OK
	rs312481 G>A			Intron 3	131	26	3	160	0.900	0.100	OK
	rs3774425 G>A			Intron 3	73	72	16	161	0.677	0.323	OK
	rs3774426 C>T			Intron 3	118	35	7	160	0.847	0.153	OK

*Genetic polymorphisms of *CACNA1C* were firstly screened in 48 randomly chosen hypertensive subjects, and then representative polymorphisms were genotyped in 161 patients with essential hypertension who were treated with L-type CCBs.
Data in parentheses ( ) based on genotyping results for *CACNA1C*.
Based on the sequencing result, the apparent LD, defined by $r^2>0.5$, was indicated by a-l.
The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (Hum. Mut., 11, 1–3, 1998).
The nucleotide number was according to the reference sequences GenBank Accession ID: NT_009759.15.
Sequence for promoter region, exon 44, and exon 47 of *CACNA1C* was abortive.
**Common SNPs of *CACNA1D* were chosen from JSNP database and genotyped in 161 patients with essential hypertension who were treated with L-type CCBs.
PCR, polymerase chain reaction; LD, linkage disequilibrium; SNPs, single nucleotide polymorphisms. Other abbreviation see in Table 1.

nitrendipine (2.5%), barnidipine (1.2%), cilnidipine (1.2%), and efonidipine (0.6%) (**Table 1**). Patients who could achieve a SBP reduction greater than 20 mmHg or a DBP reduction greater than 10 mmHg after taking L-type dCCBs were defined as responders, and those who could not were defined as nonresponders. These criteria are often used in the clinical trial of new antihypertensive drugs in Japan.

DNA Studies

Direct Sequencing for Detection of Polymorphisms in CACNA1C Genomic DNA was extracted using an NA-3000 nucleic acid isolation system (Kurabo, Osaka, Japan) and stored at –80°C until use. Human *CACNA1C*, located on chromosome 12 at p13.3, consists of 47 exons. We sequenced 48 samples from Japanese patients with HT, using a direct sequencing method described previously[21] Briefly, all exons with their flanking sequences and approximately 1,000 bp of the upstream region of exon 1, which would include promoter regions of *CACNA1C*, were individually amplified with the polymerase chain reaction (PCR) and sequenced with a ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, CA, USA). We failed to sequence the promoter region and exons 44 and 47 of *CACNA1C* because of amplification problems by the PCR. Information on the primers and the PCR conditions is available on request. The polymorphisms were identified with Sequencer software (Gene Codes Corporation, Ann Arbor, MI, USA), followed by visual inspection.

Genotyping of Polymorphisms The TaqMan-PCR method was used for genotyping sequence-proven genetic

Table 3. Genotype Distribution Between Responders and Nonresponders Treated With L-Type CCBs

Gene	SNP	R=ΔDBP >10 mmHg					R=ΔSBP >20 mmHg				
		Genotype	R	NR	χ^2	P value	Genotype	R	NR	χ^2	P value
CACNA1C	527974G>A	GG	0	8	4.501	0.105	GG	0	8	4.418	0.110
		GA	23	39			GA	22	40		
		AA	33	58			AA	26	65		
		GG	0	8	4.490	0.034	GG	0	8	3.576	0.059
		GA+AA	56	97			GA+AA	48	105		
		GG+GA	23	47	0.202	0.653	GG+GA	22	48	0.154	0.694
		AA	33	58			AA	26	65		
		OR 1.163, 95%CI 0.603–2.242					OR 0.873, 95%CI 0.442–1.722				
CACNA1D	rs312481G>A	GG	51	80	5.291	0.071	GG	45	86	11.571	0.003
		GA	4	22			GA	1	25		
		AA	1	2			AA	2	1		
		GG	51	80	4.910	0.027	GG	45	86	6.516	0.011
		GA+AA	5	24			GA+AA	3	26		
		OR 0.327, 95%CI 0.117–0.911					OR 0.221, 95%CI 0.063–0.768				
		GG+GA	55	102	0.004	0.951	GG+GA	46	111	1.957	0.162
		AA	1	2			AA	2	1		
		OR 0.927, 95%CI 0.082–10.457					OR 4.826, 95%CI 0.427–54.544				
	rs3774426C>T	CC	48	70	6.705	0.035	CC	40	78	3.616	0.164
		CT	6	29			CT	6	29		
		TT	2	5			TT	2	5		
		CC	48	70	6.370	0.012	CC	40	78	3.253	0.071
		CT+TT	8	34			CT+TT	8	34		
		OR 0.343, 95%CI 0.146–0.805					OR 0.459, 95%CI 0.194–1.084				
		CC+CT	54	99	0.133	0.715	CC+CT	46	107	0.007	0.933
		TT	2	5			TT	2	5		
		OR 0.733, 95%CI 0.138–3.907					OR 0.930, 95%CI 0.174–4.972				

ΔDBP=DBP (before treatment)-DBP (after treatment); ΔSBP=SBP (before treatment)-SBP (after treatment). Other abbreviations see in Tables 1,2.

polymorphisms of *CACNA1C* and common SNPs of *CACNA1D* chosen from the db SNP database (http://www.ncbi.nlm.nih.gov/SNP/). For sequence-proven genetic polymorphisms, polymorphisms with a minor allele frequency greater than 5% (common polymorphism) were considered candidates for genotyping. We chose a representative common SNP for genotyping among SNPs showing strong LD with an r-square greater than 0.5. Because a missense mutation may cause a direct functional change of the α1c subunit, 2 missense mutations of *CACNA1C* with a minor allele frequency less than 5% were also subjected to genotype analysis. For genetic polymorphisms of *CACNA1D* chosen from the db SNP database, 5 common SNPs (rs219847 G>A, rs312481 G>A, rs3774414 C>T, rs3774425 G>A, rs3774426 C>T) with a minor allelic frequency greater than 5% were chosen for genotyping. There was no tight LD with an r-square greater than 0.5 among these 5 SNPs in *CACNA1D*. As a consequence, 11 SNPs for *CACNA1C* and 5 SNPs for *CACNA1D* in 161 Japanese patients with HT treated with L-type dCCBs were subjected to genotype analysis. We did not perform haplotype analysis because of the study design. We evaluated the synergistic effects of SNPs associated with the effect of CCBs.

Statistical Analysis

Values are expressed as means±SD. Hardy-Weinberg equilibrium was assessed with χ^2 analysis. The overall distribution of alleles was analyzed with χ^2 analysis. The distribution of genotypes between responders and nonresponders was analyzed with 2×2 contingency tables and 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 with ANOVA followed by the Fisher protected least-significant difference test using Stat-View version 5.0 (SAS Institute Inc, Cary, NC, USA).

Results

Group Characteristics

Overall, both SBP and DBP were significantly reduced after treatment with L-type dCCBs (**Figure**). **Table 1** shows the characteristics of responders and nonresponders. When responder was defined as a SBP reduction >20 mmHg, 48 patients were defined as responders and 113 as nonresponders. When responder was defined as a DBP reduction >10 mmHg, 56 patients were responders and 105 were nonresponders. Neither sex nor body mass index showed a significant difference between responders and nonresponders. Average age and the percentage receiving monotherapy differed significantly between responders and nonresponders when responder was defined as a SBP reduction >20 mmHg. The BP before treatment with dCCBs was significantly higher in responders than in nonresponders. After treatment with dCCBs, the average BP in responders was markedly decreased; however, the average BP in nonresponders was significantly higher than that in responders. Heart rate did not differ significantly between responders and nonresponders before or after treatment with dCCBs. No significant difference in the types of L-type dCCB was found between responders and nonresponders.

Detection of Genetic Polymorphisms

First, we screened for genetic polymorphisms of *CACNA1C* in 48 randomly chosen patients with HT by means of direct sequencing. As shown in **Table 2**, we identified 2 missense mutations in *CACNA1C*. Three of 48 patients had a G-to-A substitution at nucleotide 632652 in

Table 4. Selected Genotype Interactions on the Effects of L-Type CCBs

Comparison	Positively-related polymorphisms			Number	ΔSBP	ΔDBP	P1	P2
	CACNA1C 527974G>A	*CACNA1D* rs312481G>A	*CACNA1D* rs3774426C>T					
2-way interaction								
1	AG+AA	GG	Any	124	15.2±21.1	9.9±9.9	0.0109	0.0007
	Any others			36	5.4±15.9	3.9±6.3		
2	AG+AA	Any	GG	112	13.6±22.3	10.1±10.2	0.5651	0.0031
	Any others			48	11.6±15.2	5.3±6.9		
3	Any	GG	GG	113	14.6±21.0	9.8±10.1	0.1098	0.0136
	Any others			46	8.9±18.7	5.7±7.3		
3-way interaction								
4	AG+AA	GG	GG	107	14.9±21.5	10.3±10.1	0.0801	0.0013
	Any others			52	8.9±17.6	5.2±7.2		

P1, comparison of ΔSBP between genotype groups; P2, comparison of ΔDBP between genotype groups. Other abbreviations see in Tables 1,2.

exon 45, leading to an Arg-to-Gln substitution at position 1910 (R1910Q). One patient had a G-to-A substitution at nucleotide 635110 in exon 46, leading to a Gly-to-Ser substitution at position 2004 (G2004S). Both missense mutations were found in heterozygous form. In addition, we identified 5 synonymous variations (395458G>A in exon 4, 531910C>T in exon 17, 539757G>A in exon 19, 558409C>T in exon 29, 626151G>A in exon 43) encoded for A174 (minor allelic frequency, 0.052), for D812 (0.333), for A879 (0.010), for F1262 (0.415), and for T1787 (0.402). Thirty-one additional variations in the intron and 3'-untranslated regions were also detected. As described in the Methods section, we finally chose 11 genetic polymorphisms of *CACNA1C* and 5 common SNPs of *CACNA1D* for genotype analysis in 161 patients with EHT who were treated with L-type dCCBs (**Table 2**). We failed to genotype 638741-638742insT of CACNA1C because of incomplete discrimination of the genotyping signals. We did not identify 635110G>A (G2004S) of *CACNA1C* in the 161 samples. The allelic frequencies of another 8 SNPs of *CACNA1C* determined with genotyping were similar to those identified with direct sequencing.

Association Study for the Effect of L-Type dCCBs

The clinical characteristics of patients with the 632652G>A (R1910Q) mutation did not show any specific clinical features after treatment with L-type dCCBs (data not shown). Thus, 8 common SNPs of *CACNA1C* and 5 of *CACNA1D* subjected to genotype analysis were used to study their relationship to the effects of L-type dCCBs. Control for deviation from Hardy-Weinberg equilibrium yielded nonsignificant results in all SNPs examined in this study. On basis of a comparison of each allele frequency between responders and nonresponders, 1 of *CACNA1C*, 527974G>A, and 2 SNPs of *CACNA1D*, rs312481G>A and rs3774426C>T, showed significant correlations with the effects of L-type dCCBs (**Table 3**). When a response was defined as a DBP reduction >10 mmHg, the prevalence of *CACNA1C* 527974G>A differed significantly in the dominant model, in that *CACNA1D* rs3774426C>T differed in the additive and recessive models, and that of *CACNA1D* rs312481G>A differed only in the recessive model. When a response was defined as a SBP reduction >20 mmHg, the prevalence of *CACNA1D* rs312481G>A significantly differed in the additive and recessive models. *CACNA1C* 527974G>A and *CACNA1D* rs3774426C>T showed a marginal relation to the effects of L-type dCCBs.

Figure show the comparison of BP in the dominant or recessive model in 3 SNPs that were significantly associated with the effect of L-type dCCBs shown in **Table 3**. The basal SBP and DBP were significantly reduced by treatment with L-type dCCBs in patients with GG carriers in *CACNA1D* rs312481G>A or CC carriers in rs3774426C>T, with GA+AA carriers in *CACNA1C* 527974G>A, and also with CT+TT carriers in *CACNA1D* rs3774426C>T. After treatment with dCCBs, DBP in patients with GG in rs312481G>A, with CC in rs3774426C>T, and with GA+AA in *CACNA1C* 527974G>A was significantly reduced when compared with patients with other allele carriers (P=0.0126 for rs312481G>A, 0.0283 for rs3774426C>T, and 0.0108 for 527974G>A) (**Figure**). Patients with GG carrier in rs312481G>A also showed a significant reduction in SBP after treatment with L-type dCCBs when compared with patients with GA+AA carrier (P=0.0101). Both SBP and DBP were significantly decreased by treatment with dCCBs in patients with GG carrier in *CACNA1D* rs312481G>A, but there was no significant reduction in BP in GA+AA in *CACNA1D* rs312481G>A. In contrast, significant differences in the antihypertensive effect on either SBP or DBP of treatment with dCCBs between alleles were not seen in *CACNA1D* rs3774426C>T or *CACNA1C* 527974G>A.

The genotype interactions on the effects of L-type dCCBs are shown in **Table 4**. When interactions between 2 polymorphisms were analyzed, a much greater reduction in DBP after treatment with dCCBs was observed for the simultaneous presence of *CACNA1C* 527974 GA+AA–*CACNA1D* rs312481 GG or *CACNA1C* 529874 GA+AA–*CACNA1D* rs3774426 CC. The 3-way interaction models also showed a much greater reduction in DBP for the simultaneous presence of *CACNA1C* 527974 GA+AA–*CACNA1D* rs312481 GG–*CACNA1D* rs3774426 CC.

Discussion

The present study has demonstrated that *CACNA1C* 527974G>A, *CACNA1D* rs312481G>A, and *CACNA1D* rs3774426C>T are associated with the antihypertensive effects of L-type dCCBs in Japanese patients with EHT. In particular, the greatest sensitivity to the effects of dCCBs was observed with *CACNA1D* rs312481G>A, which showed a significant association with the effects of L-type dCCBs in the reduction of both SBP and DBP. A patient with HT and GA+AA in *CACNA1D* rs312481G>A or with GG in *CACNA1C* 527974G>A is predicted to be a nonresponder to

L-type dCCBs (**Table 3, Figure**). In addition, there was a synergistic effect between the genetic polymorphisms of *CACNA1C* and *CACNA1D* on the lowering BP by L-type dCCBs (**Table 4**). The L-type α1c subunit plays a central role in regulating cardiac function and BP[22,23] and is a target of the L-type dCCBs widely used in the treatment of HT.[24] Therefore, we speculated that genetic polymorphisms of *CACNA1C* might be related to the effects of L-type dCCBs. In this study, we demonstrated that 527974G>A of *CACNA1C* has a significant association with the effects of L-type dCCBs. While we were preparing this report, Bremer et al reported that *CACNA1C* polymorphisms are associated with the efficacy of dCCBs in the treatment of HT in white subjects.[25] The results of both studies suggest that genetic polymorphisms of *CACNA1C* influence the effects of L-type dCCBs in patients with HT; however, how these genetic polymorphisms affect the effects of L-type dCCBs is still unknown. Because 527974G>A is located in intron 13, this SNP itself might not influence α1c function. Although we could not find functional polymorphisms linked with 527974G>A in our results or in HapMap data for Japanese, there may be functional polymorphisms in the promoter region (which we failed to sequence) or genes adjacent to *CACNA1C*. In addition, human *CACNA1C*, spanning >500kb, maps to chromosome 12p11.2 and undergoes extensive mRNA splicing, leading to numerous isoforms with different functions in altering electrophysiology properties,[26–28] affinity to DHPs,[29,30] and loss of channel functions.[31] Alternative splicing is regulated by multiple factors, including the 5' splice site, the 3' splice site, the branch site and the Py tract, as well as the intronic or exonic splicing enhancer and silencer.[31] Identifying genetic polymorphisms that affect splicing has proven difficult, as they can be located not just in the splice regions but anywhere in the large intron. Therefore, we could not rule out the possibility that 527974G>A, as well as polymorphisms linked with it in intron regions, might influence *CACNA1C* mRNA splicing.

The present study is the first to demonstrate that genetic polymorphisms of *CACNA1D* might be associated with the effects of L-type dCCBs in patients with EHT. Of the 3 SNPs that were identified to be associated with the effects of L-type dCCBs in the present study, *CACNA1D* rs312481G>A was the most strongly associated. Patients with GG homozygous for rs312481G>A were more sensitive to the effects of L-type dCCBs for reducing DBP and SBP than were patients with the GA+AA genotype. *CACNA1D* rs3774426C>T also showed a significant association with the effects of L-type dCCBs for reducing DBP. A previous study has shown that α1D does not mediate the contractility of ventricular muscle or aortic smooth muscle.[18] In addition, all L-type calcium channels studied to date are sensitive to L-type dCCBs.[19,32] However, α1D-containing L-type calcium channels appear to be significantly less sensitive to L-type dCCBs.[19,32] Therefore, how the genetic polymorphisms of *CACNA1D* affect the L-type dCCBs reduction of BP would be very interesting to know. Importantly, recent studies have shown that the lower sensitivity of α1D-containing L-type calcium channels to L-type dCCBs becomes even more significant when membrane potentials are hyperpolarized and α1c-containing L-type calcium channels are not open. The α1D-containing L-type calcium-channel current that remains in the presence of DHPs takes on the profile of an inactivating current with barium as the charge carrier.[32] This is consistent with the state-dependent nature of the blockade by DHPs.[33,34] In the presence of L-type dCCBs, α1D-containing L-type calcium channels generate low-threshold, drug-resistant, inactivating currents that resemble the R-type current of many neurons or the T-type current of sinoatrial node cells and control physiological processes, such as diastolic depolarization in sinoatrial node cells and neurotransmitter release and neuronal excitability. Because the neuroendocrine system and pacemaking may play important roles in regulating BP, variations of *CACNA1D* may influence the effects of L-type dCCBs through a change in the sensitivity of α1D-containing L-type calcium channels to L-type dCCBs. *CACNA1D* rs312481G>A and rs3774426C>T are both in intron regions. We did not find functional polymorphisms linked with them in HapMap data for Japanese (data not shown). The α1D subunit also undergoes extensive mRNA splicing, which may lead to numerous isoforms with different functions.[35,36] Whether *CACNA1D* rs312481G>A and rs3774426C>T or polymorphisms linked with them in intron regions influence *CACNA1D* mRNA splicing needs to be clarified.

Our data also show a possible synergistic effect of genetic polymorphisms of *CACNA1C* and those of *CACNA1D* on L-type dCCBs treatment in patients with EHT. This result suggests that α1D-containing and α1c-containing L-type calcium channels might coordinate the regulation of BP under physiological conditions or the responsiveness to treatment with L-type dCCBs under pathological conditions. Further functional studies are needed to clarify this point.

There is a question as to whether the contributions of *CACNA1D* rs312481G>A and rs3774426C>T and of *CACNA1C* 527974G>A to the effects of L-type dCCBs are an L-type CCB-specific finding. We speculate that the contribution of these 3 SNPs to the antihypertensive effects of L-type dCCBs is in fact dCCB-specific, because these SNPs also showed a significant association with the effects of L-type dCCBs in a study of patients who received only L-type dCCB monotherapy, despite a small sample size (data not shown).

Study Limitations

The present study was retrospective design and had a small sample size. The study subjects included not only patients receiving monotherapy with L-type dCCBs, but also those receiving combined therapy with L-type dCCBs and other antihypertensive drugs. We do not believe that this issue greatly affects the relationship between the 3 SNPs and the effects of L-type dCCBs, because the percentages of patients receiving monotherapy with L-type dCCBs and of patients receiving different L-type dCCBs, such as amlodipine and nifedipine, did not differ significantly between each allele of these SNPs. In addition, the SNPs also showed a significant association with the effects of L-type dCCBs in a study that examined only patients who had received amlodipine therapy (data not shown). However, a large-scale, prospective, controlled study of L-type dCCBs is needed to confirm the importance of these SNPs in the antihypertensive effects of L-type dCCBs. Furthermore, the BP before treatment is an important factor in the effects of antihypertensive drugs. In the present study, both SBP and DBP before treatment with L-type dCCBs were significantly higher in responders than in nonresponders. However, the BP before treatment with L-type dCCBs did not differ significantly between dCCB-sensitive and dCCB-insensitive genotypes in *CACNA1D* rs312481G

>A and rs3774426C>T and in *CACNA1C* 527974G>A when a response was defined as a change in SBP>20 mmHg or in *CACNA1D* rs3774426C>T and *CACNA1C* 527974G>A when a response was defined as a change in DBP>10 mmHg (**Table 3**). In addition, age and aging may influence the effects of antihypertensive drugs because of higher SBP and slower metabolism of dCCBs (compared with younger patients)? However, there was no significant difference in the average age of patients with dCCB-sensitive or -insensitive genotypes. Finally, regarding the statistical approach, the Bonferroni method was not performed, although multiple SNPs were investigated in the present study. No SNPs were significantly associated with the effects of L-type dCCBs according to Bonferroni criteria (P=0.05/13 SNPs, P<0.005). Although this correlation might be considered weak for this type of genetic research, we consider these 3 SNPs to be prominent candidates related to the effectiveness of L-type dCCBs, because both *CACNA1C* and *CACNA1D* have been suggested to play important roles in the effectiveness of L-type dCCBs in patients with EHT, as mentioned earlier.

In summary, rs312481G>A and rs3774426C>T of *CACNA1D* and 527974G>A of *CACNA1C* are believed to be genetic polymorphisms that confer sensitivity to the antihypertensive effects of L-type dCCBs in patients with EHT. Because association studies are not consistently reproducible, as a result of false-positive and false-negative results[37] the association of these polymorphisms with the effects of L-type dCCBs should be re-examined in other populations. These genetic polymorphisms may be useful for predicting the sensitivity of patients to treatment with L-type dCCBs and may lead to individualized therapies for HT based on genetic background.

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高血圧のテーラーメイド医療の展望

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高血圧は多因子疾患であるため，これまで数多くなされてきた高血圧の原因遺伝子の探索は非常に困難であった．しかしながら生活習慣の改善への反応にかかわる遺伝子や降圧薬関連遺伝子多型などを明らかにすることで高血圧診療の現場で遺伝子情報を用いたテーラーメイド診療をおこなえる可能性がある．本稿では高血圧テーラーメイド診療実現への展望を述べる．

はじめに

ポストゲノム時代を迎えた当初から，一塩基多型（single nucleotide polymorphisms：SNP）を解析することによって高血圧の発症を予測し，治療薬の選択をおこなうテーラーメイド医療の確立に期待がかけられてきた．わが国でも2000年より5年計画で開始された癌，高血圧，糖尿病，痴呆，喘息に対するテーラーメイド医療の確立とゲノム創薬を目標に掲げた遺伝子解析計画，ミレニアム・ゲノム・プロジェクト（MGP）が2005年3月末に予定期間を終了したが[1]，残念ながら高血圧診療におけるテーラーメイド医療はいまだに実現できていないのが現状である．その原因は高血圧が遺伝因子以外の多くの因子，とくに年齢，性別，食物，肥満，精神的ストレスなど環境要因にも影響を受けやすい多因子疾患であること，血圧という表現型が変動性の大きいもので人的に定めた140/90 mmHg以上が高血圧といった定義しかないこと，さらには原因となる遺伝的素因を有していてもすべての症例で高血圧が発症するとはかぎらず，遺伝浸透率が低いことなどがその理由である．事実，2007年，高血圧を含む7つの疾患それぞれ約2,000人と共通の正常コントロール者3,000人を対象とし，DNAマイクロアレイによる50万SNPを検討するゲノム網羅的関連解析（genome-wide association study：GWAS）の結果が英国より発表されている[2]．これによるとクローン病や1型・2型糖尿病ならびに関節リウマチなどでは，$p<10^{-5}$を示す大変強い関連性をもつSNPが検出されたが，高血圧ではこのように強い関連性を示したSNPは見出されていない．このことは前述したように高血圧における関連遺伝子を同定することのむずかしさを示した結果となっている．こういった現況ではあるが，MGPを機にゲノム研究の基盤は整備され，得られた膨大なゲノム情報はここ数年のうちに高血圧領域においても臨床の現場に応用されていくことは間違いないと考えられる．さまざまな形で遺伝子情報を用いた高血圧テーラーメイド診療の可能性が考えられる．①高血圧素因遺伝子多型を用いた高血圧発症前診断，②高血圧臓器障害関連遺伝子多型を用いた臓器障害，心血管疾患・腎障害の発症予測，③減塩など生活習慣改善療法への反応性の予測，

KEY WORDS

一塩基多型（SNP），ミレニアム・ゲノム・プロジェクト（MGP），ゲノム網羅的関連解析（GWAS），ファーマコゲノミクス

④降圧薬関連遺伝子多型を用いた降圧薬の選択などが検討されている．なかでも最も早期に実現が期待されるのは，遺伝子情報を用いて降圧薬の選択をおこなう高血圧テーラーメイド診療の確立である．われわれは，国立循環器病センターにおいておこなわれたMGPならびにその後のポストミレニアム研究において降圧薬のファーマコゲノミクスを重視して研究をおこなってきた．

本稿では，その成果も含めて高血圧のテーラーメイド診療の確立の可能性につき述べる．

1．高血圧素因の遺伝子診断

高血圧の遺伝子多型研究のはじまりは1992年のアンジオテンシノーゲン遺伝子多型（*AGN* M235T）と高血圧[3]，ならびにACE遺伝子（*ACE* I/D）と心筋梗塞の関連性[4]が報告されてからスタートした．*ACE* I/Dは日本人男性の高血圧に関連することが吹田研究大規模一般住民を対象にした研究で明らかとなり[5]，高血圧素因遺伝子としても注目されるようになった．これらのレニン・アンジオテンシン系関連遺伝子多型を用いた高血圧テーラーメイド診療が期待されてきた．現在，一部の検査メーカーや健診施設が消費者直結型（direct to consumer：DTC）の形で*ACE* I/Dなどのタイピングをしている場合があるが，うまく機能していないのが現状である．

その原因として，高血圧素因遺伝子多型を臨床検査として調べることの意義が確立していないことにある．これには以下のような種々の問題が関係する．①既報の高血圧素因遺伝子多型の高血圧発症への寄与率が小さい（オッズ比で1.2～1.4程度），②研究対象集団間での再現性が低い，③本来高血圧の素因の有無を検索すべき若年期での積極的遺伝子診断が倫理的には受け入れられていない，などの点があげられる．しかしながらGWASを含めて多くの高血圧素因遺伝子の同定を目指した研究が施行されており，今後より強力な関連性を有する遺伝子多型が明らかになって来る可能性はある．また腎血管性高血圧や原発性アルドステロン症などの頻度の多い二次性高血圧への遺伝素因の関与は明らかにされていないが，弧発例での遺伝子の関与は考えられるので，今後これらの病気の関連遺伝子多型，変異が明らかになれば，疾患の診断への応用が期待される．

2．高血圧合併症の遺伝子診断

同じ高血圧の重症度でも，患者個々で臓器障害や合併症の発症の程度には違いがある．これには個々の患者の遺伝素因が関係している可能性があるため，種々の高血圧臓器障害や合併症関連の遺伝子多型の研究が進んでいる．もし遺伝子診断で高血圧臓器障害の進行をある程度でも予測できれば，たとえガイドライン上のリスクが低リスク群と判断されても，中等あるいは高リスクに準じた高血圧治療，降圧目標の設定，降圧薬の選択をおこなうといった将来の臓器障害の発症を予防するテーラーメイド治療の実現が考えられる．さらには臓器障害関連遺伝子多型を複数有すると臓器障害や心血管合併症が発症しやすい可能性が考えられる．われわれは遺伝子解析をおこなった約950人の高血圧患者のなかで，高血圧性心肥大（*IGF1R* 2SNPs），動脈硬化（*MMP2* 1SNP），腎障害（*ACE* I/Dほか，4SNPsのうち一つ）のリスク多型を重ねて有する高血圧患者の心血管イベント発症を検討したところ，**表1**に示すように12人中8人がメジャーな心血管疾患を発症していることがわかった[6]．これはこれらの遺伝子多型を単独でもっている場合の心血管イベント発生率と比較して明らかに高率であり，リスク遺伝子多型の集積も今後のテーラーメイド高血圧診療においては考慮すべき点である．

3．生活習慣改善に対する反応性への関与

大阪大学のKatsuyaら[7]は日本人には食塩感受性を示す遺伝子多型を有する人が欧米人より多いことを自験のデータをもとに提唱している（**図1**）．つまり日本人は食塩摂取量が多いのみならず食塩感受性が強いために高血圧になる可能性が高い可能性がある．逆に食塩制限が高血圧の発症抑制や降圧治療として有効である可能性が高い．事実，Huntら[8]はTOHP（Trials of Hypertension Prevention）phase IIにおいて高血圧のリスクアレルである*angiotensinogen* T235アレルを有する患者ではM235を有する患者よりも減塩が有効であったと報告し

表 1. 高血圧臓器障害関連遺伝子多型を複数有する患者と心血管合併症

症例	年齢	心肥大関連多型 IGF1R C-328T	心肥大関連多型 IGF1R A275124C	動脈硬化関連多型 MMP2 A26223C	腎障害関連多型	心血管イベント	
					ACE I/D	3/3	
1	56	CC	AA	CC	DD	Stroke	
2	67	CC	AA	CC	DD	AMI	
3	77	CC	AA	CC	DD	AMI	
					MLR C850G	1/2	
4	76	CC	AA	CC	CC	Stroke	
5	56	CC	AA	CC	CC	None	
					SOD3 C-1708T	1/2	
6	86	CC	AA	CC	CT	Effort AP	
7	58	CC	AA	CC	CT	None	
					ECE1 T65251C	2/2	
8	56	CC	AA	CC	GG	Stroke	
9	60	CC	AA	CC	GG	Stroke	
					NPR1 G2979C	1/3	
10	71	CC	AA	CC	GC	DAA	
11	69	CC	AA	CC	GC	None	
12	60	CC	AA	CC	GC	None	計 8/12

(神出計ほか, 2005[6]より改変引用)

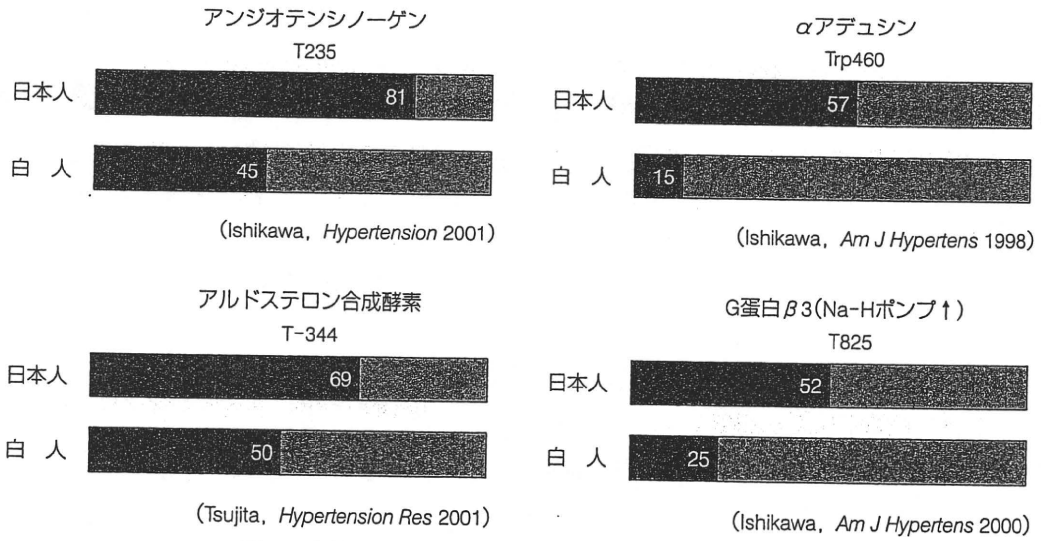

図 1. 食塩感受性関連の遺伝子多型-日本人と白人の比較
(Katsuya T *et al*, 2003[7]より改変引用)

ている．このように生活習慣改善療法にも，その人が有している遺伝子多型の違いにより反応性が違っており，遺伝情報をもとにしたテーラーメイド治療が期待される．大規模な前向き介入試験により，もっと確実な生活習慣改善の効果と遺伝子多型の関連性が明らかになることが望まれる．

4．降圧薬のファーマコゲノミクス

もっとも実現しやすく臨床的に有用性が高いと考えられるのは降圧薬関連遺伝子多型を用いて薬剤選択をおこなうテーラーメイド診療である．しかしながら肺癌治療薬のゲフィチニブの効果を上皮成長因子受容体（EGFR）の遺伝子変異で予測するといったテーラーメイド医療が癌治療の分野ではおこなわれるようになっているが，高血圧治療においてはこの方法はまだ確立していない．こ

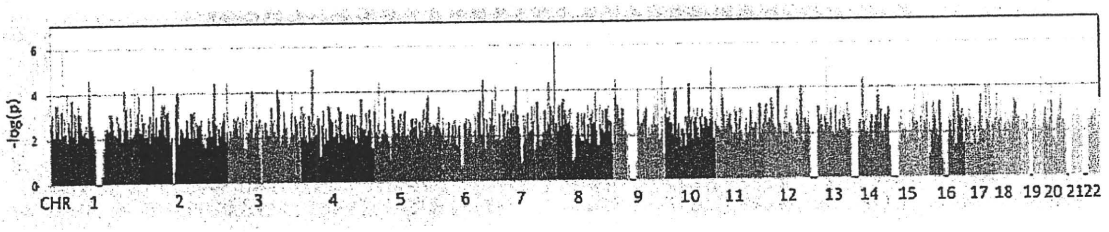

図 2. サイアザイド系利尿薬関連 SNP（GEANE より）
インダパミド投与後の血圧値を投薬前の血圧値で補正後，レスポンダー・ノンレスポンダーを判定し，GWAS をおこなった．染色体 7 番に $p<10^{-6}$ を超える最も強い関連 SNP を認めた．

れは降圧薬の効果や副作用を確実に予測できる遺伝子多型がわかっていないことが原因である．われわれは MGP 施行当時より降圧薬関連遺伝子多型を明らかにするために降圧薬のファーマコゲノミクス研究をおこなってきた．その一環でおこなわれた降圧薬感受性遺伝子多型同定のための多施設共同研究 GEANE（Gene Evaluation for ANtihypertensive drug Effect）は国立循環器病センターが中心となり全国の大学・医療センター計 24 施設とともにおこなった研究である．GEANE では同一患者にサイアザイド系利尿薬（TD）インダパミド，ジヒドロピリジン系 Ca 拮抗薬アムロジピン，ARB バルサルタンをクロスオーバー法で服用させて降圧効果を調べ，遺伝子多型はゲノム網羅的に 50 万 SNPs を検討している．最終的に 154 例の症例登録があり，最近解析結果が発表された．GEANE ではこれら 3 剤の降圧効果関連 SNP のみならず，TD 系利尿薬投与後の尿酸上昇やカリウム低下に関わる SNP も検討している．その結果の一部を**図 2** に示す．TD の効果関連で最も強い関連性を示す SNP は，染色体 7 番にあり，その他，Ca 拮抗薬，ARB 関連 SNP，さらには TD 後の尿酸上昇やカリウム低下に関連する SNP も多数明らかになった．GEANE で得られた膨大な基礎情報から，降圧薬を選択する方法を現在われわれは模索している．現在，SNP から降圧薬を選択する方法の有用性を前向きに検討する GEANE2 も準備段階であり，遺伝子情報を用いた降圧薬選択法を用いた高血圧テーラーメイド診療の実現も近いと考えている．

おわりに

テーラーメイド医療の実現に向けて

高血圧のテーラーメイド医療実現には適確な研究成果の集積と出てきた遺伝子多型を用いた迅速遺伝子診断システムの開発，このような遺伝子診断システムを導入した場合の有用性を確かめる前向き試験による遺伝子を考慮することの有用性の証明が必要と考えられる．今後は遺伝子多型診断を考慮した新しい高血圧治療ガイドラインの制定なども必要になる可能性がある．無駄が少なく，より安全で，合併症を減少させることができるような高血圧診療を患者に提供することを最終目標に研究を進めることが重要である．

謝辞
降圧薬関連遺伝子多型の前向き臨床試験は 2005～2009 年度の厚生労働科学研究費（研究代表者-国立循環器病センター部長：河野雄平）のサポートを受けている．

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血　圧

別　刷

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各種降圧薬の中心動脈圧の低下効果

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降圧薬は上腕血圧とともに中心動脈圧（中心血圧）を低下させるが，両者への効果は必ずしも同様ではない．Ca 拮抗薬，RA 系抑制薬といった血管拡張性の降圧薬は，β遮断薬や利尿薬に比較して中心血圧の降圧効果が大きいことが認められている．大規模臨床試験の成績とあわせると，中心血圧をよく下げる薬剤は心血管予後の改善効果が大きいことが示唆される．しかし，硝酸薬や直接の血管拡張薬は中心血圧への効果は大きいが，臓器保護や心血管予後への効果は明らかではない．したがって，降圧薬による心血管保護には中心血圧の低下が関連するであろうが，これのみで決定されるわけではないと考えられる．

はじめに

中心動脈圧あるいは中心血圧は，心臓に近い部位への圧負荷を反映することから，上腕血圧より高血圧性臓器障害に強く関係し，降圧治療における測定意義が提唱されている[1)~3)]．降圧薬は上腕血圧とともに中心血圧を低下させるが，両者への効果は必ずしも同様ではない．薬剤の中心血圧への影響については，以前より小規模な基礎的および臨床的研究はなされてきたが，Ca 拮抗薬とβ遮断薬を一次薬として治療効果を比較検討した大規模臨床試験 ASCOT（Anglo-Scandinavian Cardiac Outcomes Trial）のサブスタディである CAFE（Conduit Artery Function Evaluation）研究によって脚光を浴びることになった[4)]．

KEY WORDS

中心血圧，高血圧，降圧薬

本稿では，各種降圧薬の中心血圧への効果について，上腕血圧との差や薬剤間の違いを含めて概説する．

1．Ca 拮抗薬

Ca 拮抗薬は中心血圧を比較的大きく低下させることが，いくつかの臨床研究において示されている．Morgan ら[5)]は，少数例ではあるが，高齢の高血圧患者における Ca 拮抗薬，利尿薬，ACE 阻害薬，β遮断薬の効果を，プラセボ対照無作為交叉法により検討している．中心血圧は，シグモコア®を用いて測定された．Ca 拮抗薬は上腕血圧も大きく下降させたが，中心血圧の降圧も最も大きく，また上腕血圧より中心血圧への効果がやや大であった（**図 1**）．

CAFE 研究は，ASCOT 試験のサブスタディであるが，2,199 名の高血圧患者について，Ca 拮抗薬アムロジピンあるいはβ遮断薬アテノロールを中心とする治療法の中心血圧への効果を調べている[4)]．上腕血圧の降圧効果には両群間に差を認めなかったが，Ca 拮抗薬中心の治

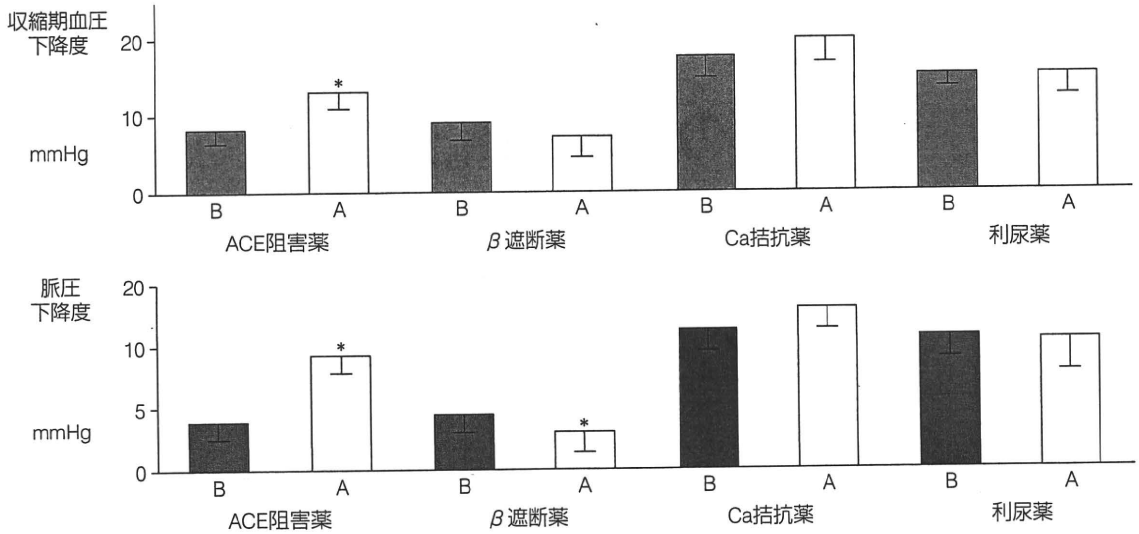

図 1. 4種の降圧薬の上腕動脈および大動脈の収縮期血圧および脈圧への効果
B：上腕動脈，A：大動脈，*両血管における効果に有意差あり．
(Morgan T *et al*, 2004[5]より引用)

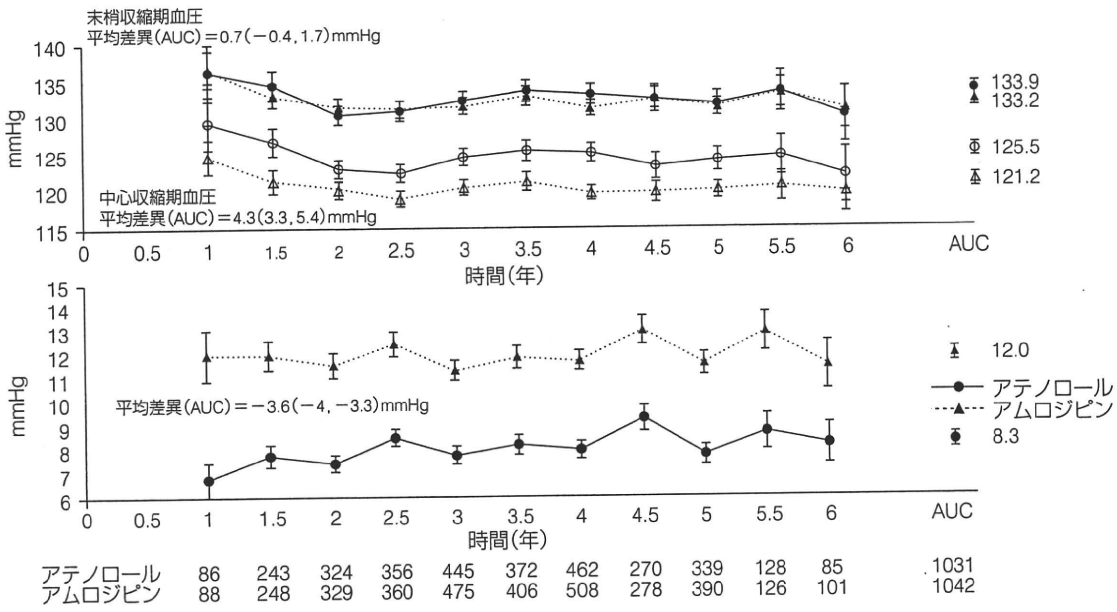

図 2. ASCOT-CAFE 研究におけるアムロジピン群とアテノロール群の上腕および中心血圧（上）およびその差（下）の推移
(Williams B *et al*, 2006[4]より引用)

療のほうが中心血圧はより低下していた（**図 2**）[4]．ASCOT の主試験においては，前者が後者より心血管合併症の発生が少なかったとの結果が得られており，両群における中心血圧の差が心血管予後に関係したことが示唆される．

わが国でも，自治医科大学や筆者らの施設を含む共同研究の ABC-J 研究（Antihypertensives and Blood pressure of Central artery study in Japan）において，各種の降圧薬の中心血圧への効果が検討されている[6]．これは観察的研究であるが，1,712 名の治療中の高血圧患者および未治療の 1,049 名について，血圧脈波検査装置（HEM-9000AI®）を用いて中心血圧を推計したものであ

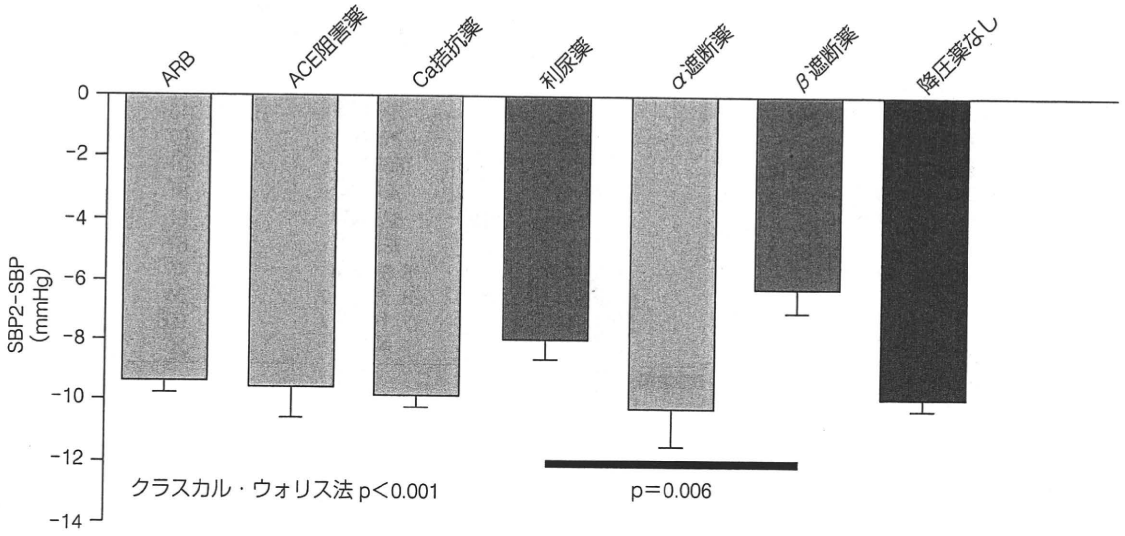

図 3. ABC-J 研究における各種降圧薬により治療中の高血圧患者および未治療者における推定中心血圧（SBP2）と上腕血圧（SBP）の差
（河野雄平ほか，2008[8]発表データ）

る．Ca 拮抗薬，RA 系抑制薬，α 遮断薬といった血管拡張性の降圧薬は，β 遮断薬や利尿薬に比較して，中心血圧の降圧効果が大きいことが認められた（**図 3**）．

Ca 拮抗薬の中心血圧への効果が大きい理由としては，末梢血管（小動脈）を拡張させることにより脈波の反射波が減弱し，augmentation index（AI）や augmentation pressure が小さくなることが，おもな機序であろうと考えられる．

2．レニン・アンジオテンシン（RA）系抑制薬

ACE 阻害薬や ARB といった RA 系の抑制薬も血管拡張作用が比較的強く，中心血圧を効果的に低下させることが認められている．

1）ACE 阻害薬

Morgan らの比較研究では，ACE 阻害薬は上腕血圧への効果は小さいが，中心血圧への効果は比較的大きいことが示されている（**図 1**）[5]．両血管における降圧効果の差は有意であった．ABC-J 研究においても，ACE 阻害薬は β 遮断薬や利尿薬に比較して，中心血圧への効果が大きかった（**図 3**）[6]．

REASON 研究（Preterax in Regression of Arterial Stiffness in a Controlled double-blind study）のサブスタディにおいては，ACE 阻害薬ペリンドプリルと利尿薬インダパミドの併用が，β 遮断薬アテノロールにくらべ，上腕血圧への効果も大きかったが，中心血圧の収縮期血圧と脈圧をよく低下させている[7]．また，前者が後者よりも左室肥大軽減をもたらし，心筋重量の変化は上腕血圧より中心血圧の変化とより関連していることが認められている．

Hirata ら[8]は，ラミプリルとアテノロールの急性効果を検討し，上腕血圧に対する中心血圧の下降度は前者が後者より大きいことを観察している．しかし，この研究では上腕血圧への効果もラミプリルがアテノロールより大であった．また，大動脈の脈波速度は同程度に低下したが，上下肢の脈波速度への効果は前者が後者より大であった．

利尿薬と比較した場合の ACE 阻害薬の中心血圧への効果については，成績が一致していないが，ACE 阻害薬がややすぐれているようである．Morgan らの研究や ABC-J 研究では，ACE 阻害薬が利尿薬より，上腕血圧と比較しての中心血圧への効果はいくらか大きかった[5][6]．一方，ACE 阻害薬と利尿薬を基礎薬とする大規模臨床試験である ANBP 試験（Australian National Blood Pressure Trial）のサブスタディでは，中心血圧お

表 1. β遮断薬服用中および非服用中の患者における血行動態指標

血行動態指標	β遮断薬なし	β遮断薬あり	p 値
上腕収縮期圧（mmHg）	144±22	146±25	.64
上腕拡張期圧（mmHg）	77±15	72±15	.13
上腕脈圧（mmHg）	66±23	75±20	.07
大動脈収縮期圧（mmHg）	125±21	140±21	.01
大動脈拡張期圧（mmHg）	77±16	78±12	.81
大動脈脈圧（mmHg）	47±19	62±20	.01
心拍数（beats/min）	71±15	73±13	.57
脈波速度（m/s）	8.5±2.6	8.9±2.0	.46
AI（%）	22.3±14	28.7±11.9	.04
AI 75（%）	20.1±11	27.7±10.7	.005
大動脈内中膜最大厚（mm）	2.4±1.2	2.8±1.6	.20

AI：augmentation index，AI 75：心拍数補正 AI
（Olafiranye O *et al*, 2008[12]より引用）

よび上腕血圧の下降度は，両群ほぼ同等であった[9]．しかし，Jiang らのラミプリルとインダパミドを比較した無作為研究においては，前者が後者より中心血圧および AI への効果が大きいことが示されている[10]．

2）ARB

ARB の中心血圧への効果を調べた研究は少ないが，ACE 阻害薬と同様に比較的大きいと考えられる．ABC-J 研究における中心血圧と上腕血圧の差は，ARB 群は ACE 阻害薬群と同等で，β遮断薬や利尿薬より中心血圧への効果が大きかった（**図 3**）[6]．

Dhakam ら[11]は，少数例の高血圧患者を対象に，ARB エプロサルタンとβ遮断薬アテノロールの効果を無作為交叉法により検討した．この研究では，上腕血圧への効果は同等であったが，中心血圧の低下度は前者が後者より大であった．一方，大動脈脈波速度の低下度は後者が前者より大きく，AI は前者で減少，後者で増加した．したがって，ARB による中心血圧低下には大血管のスティフネスの変化は寄与せず，小血管の拡張による AI 減少の関与が大きいと考えられる．

3．利尿薬

利尿薬の中心血圧への効果は，あまり大きくはない．Morgan らの研究や ABC-J 研究においては，上腕血圧との比較では，利尿薬による中心血圧の低下度は Ca 拮抗薬や RA 系抑制薬よりいくらか小さく，β遮断薬よりやや大きい（**図 1，3**）[5][6]．ACE 阻害薬との比較でも，利尿薬の効果は同等か弱いことが示されている[9][10]．

しかし，高齢高血圧患者を対象とした Morgan らの研究では，利尿薬による中心血圧の低下度自体は上腕血圧と同等であり，また ACE 阻害薬に勝るとも劣らず，β遮断薬より大きい（**図 1**）[5]．利尿薬は長期的には末梢血管抵抗を減少させることから，中心血圧に対してもかなりの効果が期待できると考えられる．

4．β遮断薬とα遮断薬

β遮断薬の中心血圧への効果は，前述したようにほかの降圧薬，とくに血管拡張性の薬剤にくらべると小さい（**図 1，3**）．β遮断薬を服用している患者と服用していない患者について検討した Olafiranye らの研究においても，β遮断薬服用者は上腕血圧は同等でも中心血圧や AI は高値であった（**表 1**）[12]．中心血圧への効果が比較的弱いことが，アテノロールを主薬とする治療を Ca 拮抗薬と比較した ASCOT や，ARB ロサルタンと比較した LIFE 試験（Losartan Intervention For Endpoint reduction in hypertension）といった大規模臨床試験において，β遮断薬が劣った理由の 1 つではないかと考えられている．

β遮断薬の中心血圧への効果が小さい理由としては，中心血圧には AI が強く関係しており，心拍数は AI の規定因子（負の）であることから[13]，心拍数減少が AI および中心血圧を比較的高くなるように働くことが推定される．実際，ABC-J 研究におけるβ遮断薬と他薬との差は，心拍数補正により減弱した[6]．しかし，Olafiranye らの研究ではβ遮断薬服用者と非服用者とのあいだに

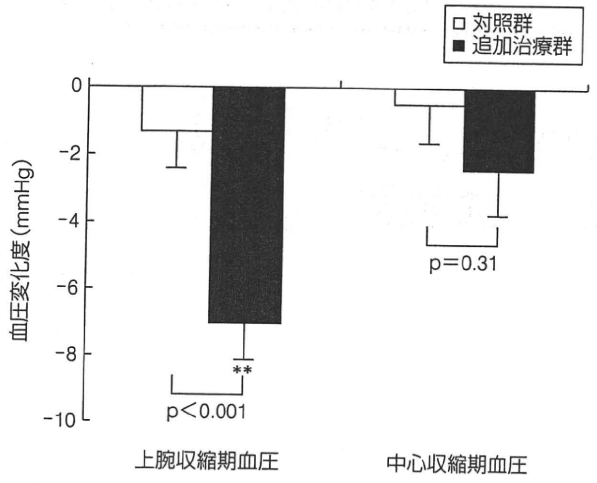

図 4. Japan Morning Surge 1 研究における α 遮断薬（および β 遮断薬）による治療強化群および対照群の上腕および中心血圧の変化
（Matsui Y *et al*, 2008[14] より引用）

心拍数の差はなく（**表 1**）[12]，他の要因も関与すると考えられる．また，β 遮断薬には多くの種類があり，心拍数への影響が小さいものや，血管拡張に働くものもある．これらの β 遮断薬は中心血圧にも異なった効果を示すと考えられる．

α 遮断薬の中心血圧への効果を調べた研究は少ないが，ABC-J 研究においては，α 遮断薬は Ca 拮抗薬，RA 系抑制薬と同様に，比較的大きい効果が認められた（**図 3**）[6]．しかし，降圧治療中で早朝高血圧を呈する患者に α 遮断薬ドキサゾシンを投与した（不十分な場合にはアテノロールを追加）Japan Morning Surge 1 研究では，上腕血圧はよく下がったのに対し，中心血圧への効果は小さいことが示されている（**図 4**）[14]．これらの差の理由は明らかではないが，β 遮断薬の併用のみではなさそうであり，さらに検討を要すると考えられる．

5．その他の血管拡張薬

硝酸薬は降圧薬としてはあまり用いられないが，強力な血管拡張作用を有しており，ニトログリセリンは上腕動脈圧より中心動脈圧を大きく低下させることが示されている[15]．ヒドララジンなどの血管拡張薬にも，同様の効果が得られるであろう．

しかし，これらの血管拡張薬は反射性の交感神経系や RA 系の活性化をもたらし，中心血圧の低下がそのまま心血管保護になるかどうかは疑わしい．硝酸薬の長期の心血管保護効果は，臨床的には明らかでない．ヒドララジンは主要降圧薬としては用いられず，実験的には，血圧を下げるが臓器保護効果に乏しい薬として他薬の対照に用いられている．

おわりに

各種の降圧薬の中心血圧への効果について述べた．Ca 拮抗薬，RA 系抑制薬といった血管拡張性の降圧薬は，β 遮断薬や利尿薬に比較して中心血圧の降圧効果が大きいことが認められており，α 遮断薬については結果が一致していない．最近の大規模臨床試験の成績と合わせて，中心血圧をよく下げる薬剤は心血管予後の改善効果が大きいことが示唆されている．しかし，降圧薬の心血管保護効果は中心血圧の低下だけで決まるものではないであろう．この領域におけるさらなる研究の進展と知見の集積が期待される．

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Comparison between carotid-femoral and brachial-ankle pulse wave velocity as measures of arterial stiffness

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Background **Arterial stiffness is an important risk factor for cardiovascular disease. Carotid-femoral pulse wave velocity (cfPWV) is the most recognized and established index of arterial stiffness. An emerging automatic measure of PWV primarily used in the Asian countries is brachial-ankle PWV (baPWV).**

Method **To systematically compare these two methodologies, we conducted a multicenter study involving a total of 2287 patients.**

Results **There was a significant positive relation between baPWV and cfPWV ($r = 0.73$). Average baPWV was approximately 20% higher than cfPWV. Both cfPWV and baPWV were significantly and positively associated with age ($r = 0.56$ and 0.64), systolic blood pressure ($r = 0.49$ and 0.61), and the Framingham risk score ($r = 0.48$ and 0.63). The areas under the receiver operating curves (ROCs) of PWV to predict the presence of both stroke and coronary artery disease were comparable between cfPWV and baPWV.**

Conclusion **Collectively, these results indicate that cfPWV and baPWV are indices of arterial stiffness that exhibit similar extent of associations with cardiovascular disease risk factors and clinical events.** *J Hypertens* **27:2022–2027 © 2009 Wolters Kluwer Health | Lippincott Williams & Wilkins.**

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Keywords: arterial distensibility, arterial elasticity, vascular assessment

Abbreviations: AUC, area under the curve; baPWV, brachial-ankle pulse wave velocity; CAD, coronary artery disease; cfPWV, carotid-femoral pulse wave velocity; CHD, coronary heart disease; eGFR, estimated glomerular filtration rate; PWV, pulse wave velocity; ROC, receiver operating characteristics

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Introduction

Arterial stiffness is associated with a number of deleterious cardiovascular conditions [1–3] and has been identified as an independent risk factor for cardiovascular disease [4]. Because of its clinical importance, a number of indices have been developed and introduced to characterize arterial stiffness [5–8]. However, clinicians and researchers still report great difficulties in selecting the most appropriate methodology for their specific use [7]. Parenthetically, a measure of arterial stiffness has not been fully incorporated in routine clinical practice. Although no one methodology has been proved superior, pulse wave velocity (PWV) is the most recognized and established index of arterial stiffness [7]. The most frequently studied index to date among a variety of PWV measures is carotid-femoral PWV (cfPWV). cfPWV has been used in landmark studies of arterial stiffness conducted in Europe [2,9] and Australia [10] as well as in the Framingham Heart Study in the USA [11]. Despite the accumulating clinical evidence, this measure of PWV has not been fully included in routine clinical settings. An emerging measure of PWV that has been widely used in Japan and other east-Asian countries in the past 10 years is brachial-ankle PWV (baPWV) [8,12–15] (or some have referred to as brachial-ankle PWV index [14]). This automated measure of PWV is very unique in that it has been widely used in routine clinical settings, at least in Japan, with impressive number of machines (~10 000) already been incorporated in various clinics and hospitals.

Although these two PWV measures are widely used in the Western and Eastern societies, respectively, associations between the two are not clear. A few studies that have attempted to address this issue are small scale in nature [8,13]. Additionally, it is not known how each of the arterial stiffness measures are associated with coronary heart disease (CHD) risk factors. Moreover, how both techniques are comparatively related to clinical events is not currently known. In an attempt to systematically address these issues, we conducted a multicenter study to determine associations between cfPWV and baPWV.

Methods

Patients

Patients were participants in the community-based research studies from six different institutions in Japan and one in the USA. A total of 2287 adults (1265 men and 1022 women) were studied. All procedures were reviewed and approved by the local Human Research

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Committees. Each patient provided written consent to participate in the study.

Before the experiments, patients abstained from alcohol and caffeine and fasted for at least 3 h. Patients were studied under supine resting conditions in a quiet, temperature-controlled room.

Pulse wave velocity measurements

Electrocardiogram, bilateral brachial and ankle blood pressures, and carotid and femoral arterial pulse waves were simultaneously measured with a vascular testing device (VP-2000; Omron Healthcare) [12]. This machine was originally developed as a screening device for hypertension (via blood pressure), peripheral artery disease (via ankle brachial index), and arterial stiffness (via PWV), and this necessitated the use of four blood pressure cuffs on each limb. Carotid and femoral arterial pressure waveforms were stored for 30 s by applanation tonometry sensors attached to the left common carotid artery (via a neck collar) and left common femoral artery (via elastic tape around the waist). Bilateral brachial and post-tibial arterial pressure waveforms were stored for 10 s by extremities cuffs, connected to a plethysmographic sensor and an oscillometric pressure sensor, wrapped around both arms and ankles.

Pulse wave velocity was calculated from the distance between two arterial recording sites divided by transit time. Transit time was determined from the time delay between the proximal and distal 'foot' waveforms. The foot of the wave was identified as the commencement of the sharp systolic upstroke, which was automatically detected by a band-pass filter (5–30 Hz). Time delay between right brachial and tibial arteries (Tba), between carotid and femoral arteries (Tcf), and between femoral and tibial arteries (Tfa) were obtained. The path length from the carotid to the femoral artery (Dcf) was directly assessed in duplicate with a random zero length measurement over the surface of the body with a nonelastic tape measure [16]. For patients whose distance between the carotid and femoral artery was not available, Dcf was estimated using the equation [0.318 × height (cm) + 10.56] [17]. Agreement between cfPWV obtained using the estimated Dcf and directly measured Dcf was excellent ($r = 0.99$). The path lengths from the suprasternal notch to brachial artery (Dhb), from suprasternal notch to femur (Dhf), and from femur and ankle (Dfa) were calculated automatically by the machine using the following equations [13]:

$$\text{Dhb} = (0.220 \times \text{height}\{\text{cm}\} - 2.07)$$

$$\text{Dhf} = (0.564 \times \text{height}\{\text{cm}\} - 18.4)$$

$$\text{Dfa} = (0.249 \times \text{height}\{\text{cm}\} + 30.7)$$

Pulse wave velocity was calculated by the following equations:

$$\text{Carotid–femoral PWV} = \frac{\text{Dcf}}{\text{Tcf}}$$

$$\text{Brachial–ankle PWV} = \frac{\text{Dhf} + \text{Dfa} - \text{Dhb}}{\text{Tba}}$$

The results obtained with right side and left side baPWV were identical ($r = 0.97$). As such, right baPWV is reported in the present study. The validity and reliability of the automatic device for measuring PWV have been established previously [12].

Blood samples

A blood sample was collected from the antecubital vein using venipuncture after an overnight fast. Plasma concentrations of glucose, lipids, and lipoproteins were determined by use of a standard enzymatic technique as previously described [16]. Glomerular filtration rate (eGFR) was calculated using the following equation introduced by the Japanese Society of Nephrology [18].

$$\text{Men}: \ \text{eGFR}\,(\text{ml/min per } 1.73\,\text{m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{age}^{-0.287}$$

$$\text{Women}: \ \text{eGFR}\,(\text{ml/min per } 1.73\,\text{m}^2) = 194 \times \text{Cr}^{-1.094} \times \text{age}^{-0.287} \times 0.739$$

Statistical analyses

Univariate regression and correlation analyses were used to analyze the relations between variables of interest. Forward stepwise multiple-regression analyses were used to determine the influence of central and peripheral arterial stiffness on baPWV. To do so, only variables that had significant univariate correlations with cfPWV and/or baPWV were included in the model. Receiver operating characteristic (ROC) curves for both cfPWV and baPWV were constructed, and area under the curves (AUC) was calculated. This analysis was performed in a cohort of 814 patients [36 strokes and 40 coronary artery disease (CAD)] collected in three different institutions. Statistical significance was set *apriori* at $P < 0.05$. Data are expressed as means ± SEM.

Results

Table 1 shows the clinical and biochemical characteristics as well as PWV for the patients. On average, baPWV was approximately 20% higher than cfPWV.

As demonstrated in Fig. 1, there was a significant positive relation between baPWV and cfPWV ($r = 0.73$). Subgroup analyses revealed no systematic differences between men and women or between Japanese and