Table 2 Genotype distributions and characteristics of pPAF-AH polymorphisms

Genotype	N	Age	Smoking	BMI	Systolic BP	Diastolic BP	LDL-C	HbA1c
Men								
I198T								
I/I	238	64.1	18.1	24.4	139.1	84.0	119.9	5.7
I/T	138	64.1	18.8	24.2	137.7	84.2	119.4	5.6
T/T	19	62.0	10.5	24.8	138.6	84.0	120.3	5.4
I/T+T/T (198T)	157	63.8	17.8	24.3	137.8	84.2	119.5	5.6
V279F								
V/V	258	63.9	17.8	24.2	138.2	83.7	120.0	5.6
V/F	125	64.3	18.4	24.5	139.3	85.0	119.2	5.6
F/F	12	60.9	16.7	24.7	137.3	82.2	119.8	5.5
V/F+F/F (279F)	137	64.0	18.2	24.5	139.2	84.8	119.2	5.6
A379V								
A/A	331	63.7	17.5	24.4	138.8	84.3	119.8	5.6
A/V	62	64.8	19.4	23.9	137.3	83.6	120.4	5.6
V/V	2	72.5	50.0	25.4	140.5	70.5	90.2	6.0
A/V+V/V (379V)	64	65.1	20.3	23.9	137.4	83.1	119.4	5.6
Women								
I198T								
I/I	220	66.2	5.9	23.7	144.2	84.2	128.5	5.5
I/T	102	67.1	1.0	23.3	139.7	80.6	126.6	5.5
T/T	16	65.4	6.3	22.9	140.1	85.1	128.8	5.5
I/T+T/T (198T)	118	66.9	1.7	23.3	139.7*	81.2*	126.9	5.5
V279F								
V/V	239	65.7	5.4	23.6	143.7	83.8	128.5	5.5
V/F	90	67.1	2.2	23.6	140.1	81.4	126.4	5.5
F/F	9	65.7	0.0	22.2	138.9	84.0	128.8	5.6
V/F+F/F (279F)	99	67.0	2.0	23.5	140.0	81.6	126.6	5.5
A379V								
A/A	270	66.8	4.8	23.4	142.1	83.3	128.8	5.5
A/V	67	65.3	3.0	24.2	144.6	82.3	125.4	5.5
V/V	1	59.0	0.0	29.1	144.0	75.0	81.2	6.1
A/V+V/V (379V)	68	65.2	2.9	24.3	144.5	83.1	124.8	5.5

Abbreviations: BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, hemoglobin.A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein.
Data represent mean values.
*$P<0.05$ *vs.* 198I/I.

PLA2G7 polymorphism. The allele frequencies in Japanese hypertensive patients were I allele 71.7% and T allele 28.3% (I198T), and A allele 84.7% and V allele 15.3% (A379V). A recent report indicated that the allele frequencies of these variants in hypercholesterolemic Sicilians were I allele 30.5% and T allele 69.5% (I198T), and A allele 33.9% and V allele 66.1% (A379V),[27] which are markedly different from the present results. In V279F, which has not been found in the Caucasians,[28,29] 29.3% of subjects were heterozygotes (215 patients) and 2.9% were homozygotes (21 patients). These values are similar to previously reported values in the healthy Japanese (heterozygotes 21.0–32.3%, homozygotes 0.9–4.2%),[26] suggesting that V279F may not affect the occurrence of hypertension, although more detailed studies with a case–control design are required.

Another important finding of this study is the significant association between polymorphisms of 0*PLA2G7* and carotid atherosclerosis. The values of C-IMT and the plaque prevalence were significantly different among genotypes in I198T or V279F, whereas C-IMT*max* showed no difference. As the differences in the C-IMT*max* values in each subject were relatively large compared with those in C-IMT, such discrepancies in association with polymorphisms may be observed. In our hypertensive patients, although the blood pressure was relatively well controlled, the prevalence of carotid plaques was significantly higher in subjects with 198T and 279F than in wild-type (198I/I and 279V/V) subjects in men. Furthermore, these two mutations were detected as independent factors for the occurrence of plaques even after adjustments with other atherosclerotic risk factors and as factors contributing to carotid plaques (Table 3). Several previous studies have suggested that 279F is a potential risk factor for atherosclerosis. Yamada *et al.*[14] reported that 279F was highly frequent in Japanese men with myocardial infarction (279V/F 33.0%, 279F/F 2.1%) compared with controls (279V/F 21.0%, 279F/F 2.2%). An increased occurrence of 279F was also reported in Japanese patients with stroke[13] and atherosclerotic occlusive disease.[15] The increased prevalence of carotid plaque in subjects with 279F observed in this study was consistent with these results. It is natural to conclude that these findings are caused by the loss of function of pPAF-AH, because pPAF-AH activity is completely abolished in individuals with 279F/F and suppressed in those with 279V/F.[12] Furthermore, it has been reported that age-dependent increase in pPAF-AH activity was diminished in 279V/F subjects,[30] suggesting that the reactive pPAF-AH induction caused by an increase in PAF is suppressed in subjects with 279V/F. These factors lead to the lack of an anti-inflammatory effect in response to injurious stimuli in the vascular wall and, therefore, may increase the risk of atherosclerosis.

I198T, another variant associated with the prevalence of carotid plaques, has been shown to reduce the substrate affinity of pPAF-AH, similar to A379V,[17] whereas these variants did not affect pPAF-AH activity.[27] However, the ranges of substrate (PAF) concentrations used in the previous study were far above the physiological level measured in plasma. Considering our results that A379V showed no correlation with the development of plaques, the functional abnormality of I198T and A379V may have small effects *in vivo*. In contrast, the strong LD with V279F ($D'=1.0$, $r^2=0.89$) may rather contribute to the association of I198T with carotid plaques in this study. In fact, all 21 subjects with 279F/F had the 198T/T genotype. Contrary to our results, Campo *et al.*[27] recently reported that R92H, I198T and A379V did not affect plasma PAF-AH activity and that these variants were not associated with carotid atherosclerosis in hypercholesterolemic Sicilian subjects. However, the population analyzed in their study was relatively small (190 subjects) and was not divided by gender. Furthermore, a significant LD was found between I198T and A379V, which was not observed in our subjects. These results suggest that the allele frequency and LD differ among races, which can lead to markedly different results.

Despite several reports indicating a significant correlation between pPAF-AH and atherosclerosis, the actual role of pPAF-AH on atherogenesis still remains unclear because of its complicated effect. As pPAF-AH degrades PAF and proinflammatory oxidized phospholipids, it may be a potent anti-atherogenic enzyme. In contrast, pPAF-AH also generates bioactive oxidized free fatty acids and lysophosphatidylcholine, which stimulate inflammatory reactions that could promote atherogenesis. However, previous studies using animal models of vascular injury have clearly indicated that pPAF-AH functions as an anti-atherogenic factor. The administration of a recombinant pPAF-AH reduced the size of myocardial infarctions and neutrophil infiltration in rabbits with coronary ligation.[28] Adenovirus-mediated

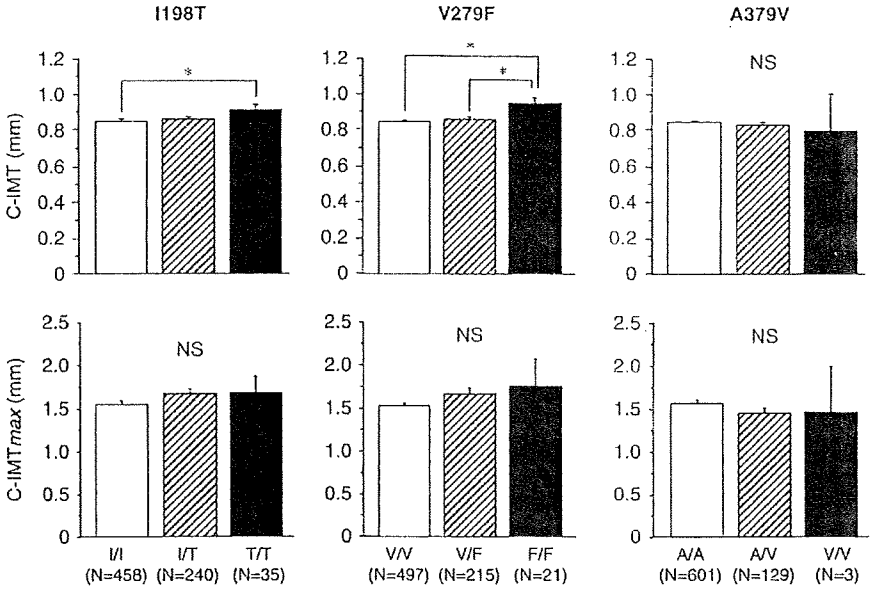

Figure 1 Comparison of mean intima-media thickness (C-IMT) or maximum IMT (C-IMT*max*) in the *PLA2G7* polymorphisms. *P*-values were evaluated with analysis of variance (ANOVA) followed by Bonferroni *post hoc* test. $^*P<0.05$. NS, not significant.

Table 3 Prevalence of carotid plaques in relation to *PLA2G7* polymorphisms

Genotype	Patients with carotid plaques/total (N)	Frequency (%)	P-value
ALL			
I198T			
I/I	266/458	58.1	
I/T	162/240	67.5	
T/T	24/35	68.6	0.014 (Trend *P*)
V279F			
V/V	288/497	57.9	
V/F	149/215	69.3	
F/F	15/21	71.4	0.004 (Trend *P*)
A/A	368/601	61.2	
A/V	82/129	63.6	
V/V	2/3	66.7	0.599 (Trend *P*)
Men			
I198T			
I/I	142/238	59.7	
I/T+T/T (198T)	111/157	70.7	0.025
V279F			
V/V	154/258	57.9	
V/F+F/F (279F)	99/137	72.3	0.014
A379V			
A/A	209/331	63.1	
A/V+V/V (379V)	44/64	68.8	0.393
Women			
I198T			
I/I	124/220	56.4	
I/T+T/T (198T)	75/118	63.6	0.201
V279F			
V/V	134/239	56.1	
V/F+F/F (279F)	65/99	65.7	0.104
A379V			
A/A	159/270	58.9	
A/V+V/V (379V)	40/68	58.8	0.992

Trend *P*-values in all subjects were evaluated with Cochran–Armitage test. Differences between groups in men or women were analyzed with χ^2-test.

pPAF-AH gene transfer prevented neointima formation in apolipoprotein E-deficient mice.[29] Hypertension may also cause inflammation of the vascular wall and induce expression of PAF, and therefore the association of the loss-of-function mutation in *PLA2G7* with carotid plaques in hypertensives supports the hypothesis that pPAF-AH is an anti-atherogenic factor. As a loss-of-function mutation such as V279F has not been identified in the Caucasians, the *PLA2G7* polymorphism may be a potential risk factor for atherosclerosis specific to the Japanese.

There are some limitations to this study. This study had a cross-sectional design, even though we analyzed a relatively large population. Our subjects were treated with antihypertensive and lipid-lowering drugs, some of which were intensively administered to patients with plaques (Table 1). These agents were reported to suppress the progression of atherosclerosis beyond their essential pharmacological effects, especially inhibitors of the renin–angiotensin system

Table 4 Logistic regression analyses of factors affecting the prevalence of carotid plaques

	Men		*Women*	
	OR (95% CI)	*P-value*	*OR (95% CI)*	*P-value*
Model 1				
198T (I/T+T/T)	1.32 (1.05–1.66)	0.016	1.15 (0.91–1.44)	0.240
279F (V/F+F/F)	1.34 (1.06–1.70)	0.011	1.20 (0.95–1.51)	0.126
Model 2				
198T	1.37 (1.08–1.74)	0.010	1.23 (0.97–1.57)	0.082
279F	1.38 (1.08–1.75)	0.009	1.31 (1.03–1.67)	0.026
Model 3				
198T	1.40 (1.10–1.79)	0.007	1.23 (0.96–1.56)	0.100
279F	1.37 (1.10–1.42)	0.006	1.30 (1.02–1.67)	0.034

Abbreviations: ACEIs, angiotensin-converting enzymes; ARBs, angiotensin II receptor blockers; BMI, body mass index; CI, confidential interval; OR, odds ratio.
Polymorphisms were analyzed by a dominant model. I198T (T/T, 1; I/T, 1; I/I, 0), V279F (F/F, 1; V/F, 1; V/V; 0).
Model 1: adjusted with age.
Model 2: adjusted with age, BMI, duration of HT, smoking, hyperlipidemia and diabetes.
Model 3: adjusted with age, BMI, duration of HT, smoking, hyperlipidemia, diabetes, ACEIs and/or ARBs, and lipid-lowering drugs.

(angiotensin II receptor blocker and angiotensin-converting enzyme inhibitor)[31,32] and statins.[33] However, even after adjusting for these factors, 198T and 279F were independently associated with plaques in men. In addition, menopausal status may influence our results in women because estrogen has been shown to increase the plasma PAF-AH activity.[34] However, we could not analyze the effect, as we did not collect the information on menopause. To further clarify these issues in detail, a cohort study in a large healthy population is required.

In conclusion, we showed the ethnic differences in allele frequency between the Caucasians and the Japanese in I198T and A379V of pPAF-AH. We also found that the loss-of-function mutation V279F in *PLA2G7* and its closely associated variant I198T are associated with carotid atherosclerosis in the similarly treated hypertensive Japanese. Our results indicated that a deficiency in pPAF-AH is a potential risk factor for atherosclerosis in hypertensive Japanese men, suggesting that a more definite risk management including blood pressure and lipid lowering is required for the hypertensive Japanese with 279F and 198T to prevent the progression of atherosclerosis.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Yoko Tokunaga and Chiyako Imai for their excellent technical assistance. We also express our gratitude to Dr Soichiro Kitamura, President of the National Cardiovascular Center, for his support in our research. This study was supported by the Program for the Promotion of Fundamental Studies in Health Sciences of the Organization for Pharmaceutical and Medical Devices Agency (PMDA) of Japan and the Ministry of Health, Labor, and Welfare of Japan.

1 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; 320: 915–924.
2 Witztum JL, Steinberg D. Role of oxidized low density lipoprotein in atherogenesis. *J Clin Invest* 1991; 88: 1785–1792.
3 Prescott SM, McIntyre TM, Zimmerman GA, Stafforini DM. Sol Sherry lecture in thrombosis: molecular events in acute inflammation. *Arterioscler Thromb Vasc Biol* 2002; 22: 727–733.
4 Karasawa K, Harada A, Satoh N, Inoue K, Setaka M. Plasma platelet activating factor-acetylhydrolase (PAF-AH). *Prog Lipid Res* 2003; 42: 93–114.
5 Blank ML, Lee T, Fitzgerald V, Snyder F. A specific acetylhydrolase for 1-alkyl-2-acetyl-sn-glycero-3-phosphocholine (a hypotensive and platelet-activating lipid). *J Biol Chem* 1981; 256: 175–178.
6 Min JH, Wilder C, Aoki J, Arai H, Inoue K, Paul L, Gelb MH. Platelet-activating factor acetylhydrolases: broad substrate specificity and lipoprotein binding does not modulate the catalytic properties of the plasma enzyme. *Biochemistry* 2001; 40: 4539–4549.
7 Tsukioka K, Matsuzaki M, Nakamata M, Kayahara H, Nakagawa T. Increased plasma level of platelet-activating factor (PAF) and decreased serum PAF acetylhydrolase (PAFAH) activity in adults with bronchial asthma. *J Investig Allergol Clin Immunol* 1996; 6: 22–29.
8 Triggiani M, De Marino V, Sofia M, Faraone S, Ambrosio G, Carratu L, Marone G. Characterization of platelet-activating factor acetylhydrolase in human bronchoalveolar lavage. *Am J Respir Crit Care Med* 1997; 156: 94–100.
9 Tetta C, Bussolino F, Modena V, Montrucchio G, Segoloni G, Pescarmona G, Camussi G. Release of platelet-activating factor in systemic lupus erythematosus. *Int Arch Allergy Appl Immunol* 1990; 91: 244–256.
10 Tselepis AD, Elisaf M, Besis S, Karabina SA, Chapman MJ, Siamopoulou A. Association of the inflammatory state in active juvenile rheumatoid arthritis with hypo-high-density lipoproteinemia and reduced lipoprotein-associated platelet-activating factor acetylhydrolase activity. *Arthritis Rheum* 1999; 42: 373–383.
11 Miwa M, Miyake T, Yamanaka T, Sugatani J, Suzuki Y, Sakata S, Araki Y, Matsumoto M. Characterization of serum platelet-activating factor (PAF) acetylhydrolase. Correlation between deficiency of serum PAF acetylhydrolase and respiratory symptoms in asthmatic children. *J Clin Invest* 1988; 82: 1983–1991.
12 Stafforini DM, Satoh K, Atkinson DL, Tjoelker LW, Eberhardt C, Yoshida H, Imaizumi T, Takamatsu S, Zimmerman GA, McIntyre TM, Gray PW, Prescott SM. Platelet-activating factor acetylhydrolase deficiency. A missense mutation near the active site of an anti-inflammatory phospholipase. *J Clin Invest* 1996; 97: 2784–2791.
13 Hiramoto M, Yoshida H, Imaizumi T, Yoshimizu N, Satoh K. A mutation in plasma platelet-activating factor acetylhydrolase (Val279–>Phe) is a genetic risk factor for stroke. *Stroke* 1997; 28: 2417–2420.
14 Yamada Y, Ichihara S, Fujimura T, Yokota M. Identification of the G994–> T missense in exon 9 of the plasma platelet-activating factor acetylhydrolase gene as an independent risk factor for coronary artery disease in Japanese men. *Metabolism* 1998; 47: 177–181.
15 Unno N, Nakamura T, Kaneko H, Uchiyama T, Yamamoto N, Sugatani J, Miwa M, Nakamura S. Plasma platelet-activating factor acetylhydrolase deficiency is associated with atherosclerotic occlusive disease in Japan. *J Vasc Surg* 2000; 32: 263–267.
16 Bell R, Collier DA, Rice SQ, Roberts GW, MacPhee CH, Kerwin RW, Price J, Gloger IS. Systematic screening of the LDL-PLA2 gene for polymorphic variants and case-control analysis in schizophrenia. *Biochem Biophys Res Commun* 1997; 241: 630–635.
17 Kruse S, Mao XQ, Heinzmann A, Blattmann S, Roberts MH, Braun S, Gao PS, Forster J, Kuehr J, Hopkin JM, Shirakawa T, Deichmann KA. The Ile198Thr and Ala379Val variants of plasmatic PAF-acetylhydrolase impair catalytical activities and are associated with atopy and asthma. *Am J Hum Genet* 2000; 66: 1522–1530.
18 Lerman LO, Nath KA, Rodriguez-Porcel M, Krier JD, Schwartz RS, Napoli C, Romero JC. Increased oxidative stress in experimental renovescular hypertension. *Hypertension* 2001; 37: 541–546.
19 Silacci P, Desgeorges A, Mazzolai L, Chambaz C, Hayoz D. Flow pulsatility is a critical determinant of oxidative stress in endothelial cells. *Hypertension* 2001; 38: 1162–1166.
20 Satoh K, Imaizumi T, Kawamura Y, Yoshida H, Takamatsu S, Takamatsu M. Increased activity of the platelet-activating factor acetylhydrolase in plasma low density lipoprotein from patients with essential hypertension. *Prostaglandins* 1989; 37: 673–682.
21 Kamide K, Takiuchi S, Tanaka C, Miwa Y, Yoshii M, Horio T, Mannami T, Kokubo Y, Tomoike H, Kawano Y, Miyata T. Three novel missense mutations of WNK4, a kinase mutated in inherited hypertension, in Japanese hypertensives: implication of clinical phenotypes. *Am J Hypertens* 2004; 17: 446–449.
22 Kamide K, Tanaka C, Takiuchi S, Miwa Y, Yoshii M, Horio T, Kawano Y, Miyata T. Six missense mutations of the epithelial sodium channel beta and gamma subunits in Japanese hypertensives. *Hypertens Res* 2004; 27: 333–338.
23 Tanaka C, Kamide K, Takiuchi S, Miwa Y, Yoshii M, Kawano Y, Miyata T. An alternative fast and convenient genotyping method for the screening of angiotensin converting enzyme gene polymorphisms. *Hypertens Res* 2003; 26: 301–306.
24 Miwa Y, Takiuchi S, Kamide K, Yoshii M, Horio T, Tanaka C, Banno M, Miyata T, Sasaguri T, Kawano Y. Identification of gene polymorphism in lipocalin-type prostaglandin D synthase and its association with carotid atherosclerosis in Japanese hypertensive patients. *Biochem Biophys Res Commun* 2004; 322: 428–433.
25 Miwa Y, Takiuchi S, Kamide K, Yoshii M, Horio T, Tanaka C, Banno M, Miyata T, Sasaguri T, Kawano Y. Insertion/deletion polymorphism in clusterin gene influences serum lipid levels and carotid intima-media thickness in hypertensive Japanese females. *Biochem Biophys Res Commun* 2005; 331: 1587–1593.
26 Karasawa K, Harada A, Satoh N, Inoue K, Setaka M. Plasma platelet-activating factor-acetylhydrolase (PAF-AH). *Prog Lipid Res* 2003; 42: 93–114.
27 Campo S, Sardo MA, Bitto A, Bonaiuto A, Trimarchi G, Bonaiuto M, Castaldo M, Saitta C, Cristadoro S, Saitta A. Platelet-activating factor acetylhydrolase is not associated with carotid intima-media thickness in hypercholesterolemic Sicilian individuals. *Clin Chem* 2004; 50: 2077–2082.

28 Morgan EN, Boyle Jr EM, Yun W, Kovacich JC, Canty Jr TG, Chi E, Pohlman TH, Verrier ED. Platelet-activating factor acetylhydrolase prevents myocardial ischemia-reperfusion injury. *Circulation* 1999; 100(Suppl II): 365–368.
29 Quarck R, De Geest B, Stengel D, Mertens A, Lox M, Theilmeier G, Michiels C, Raes M, Bult H, Collen D, Van Veldhoven P, Ninio E, Holvoet P. Adenovirus-mediated gene transfer of human platelet-activating factor-acetylhydrolase prevents injury-induced neointima formation and reduces spontaneous atherosclerosis in apolipoprotein E-deficient mice. *Circulation* 2001; 103: 2495–2500.
30 Yamada Y, Yoshida H, Ichihara S, Imaizumi T, Satoh K, Yokota M. Correlations between plasma platelet-activating factor acetylhydrolase (PAF-AH) activity and PAF-AH genotype, age, and atherosclerosis in a Japanese population. *Atherosclerosis* 2000; 150: 209–216.
31 Peters S, Gotting B, Trummel M, Rust H, Brattstrom A. Valsartan for prevention of restenosis after stenting of type B2/C lesions: the VAL-PREST trial. *J Invasive Cardiol* 2001; 13: 93–97.
32 ACE Inhibitor Myocardial Infarction Collaborative Group. Indications for ACE inhibitors in the early treatment of acute myocardial infarction: systematic overview of individual data from 100,000 patients in randomized trials. *Circulation* 1998; 97: 2202–2212.
33 Aengevaeren WR. Beyond lipids—the role of the endothelium in coronary artery disease. *Atherosclerosis* 1999; 147(Suppl 1): S11–S16.
34 Yoshimura T, Ohshige A, Maeda T, Ito M, Okamura H. Estrogen replacement therapy decreases platelet-activating factor-acetylhydrolase activity in post-menopausal women. *Maturitas* 1999; 31: 249–253.

高血圧テーラーメイド治療を目指した薬理遺伝学的アプローチ

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はじめに

高血圧症の9割以上は本態性高血圧（EHT）であり，わが国で約4,000万人の罹患者がいると考えられているように，EHTはもっとも頻度の高い生活習慣病である．さらに，多くのEHT患者は家族歴を有し，遺伝の血圧の変化に対する寄与率は30～50％あると推定されている[1]．したがって，高血圧の原因遺伝子を同定することがもたらすインパクトは，計り知れないものがあると考えられる．

しかしながら，多因子疾患であるEHTには原因遺伝子が複数存在する可能性が示唆されており[2]，現在報告されているものの多くは高血圧関連遺伝子多型である．とくに一塩基多型（single nucleotide polymorphism: SNP）はタイピングの容易さから，高速タイピングに適しており，近年，多数の検体を用いた解析に頻用され，さらにSNPタイピングによるゲノム網羅的関連解析（genome wide association study: GWAS）も多くなされるようになった．

ポストゲノム時代を迎えた当初から，SNPを解析することによって高血圧の発症を予測し，治療薬の選択を行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[3]，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限らず，遺伝浸透率が低いことなどがその理由である．

事実，一昨年，高血圧を含む七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GWASの結果が英国より発表されている[4]．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

[Key words]　高血圧，テーラーメイド医療，降圧薬
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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，ここ数年の内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．

とくにもっとも臨床的に有用性が高く，実現可能と期待されるのは，遺伝子情報を用いて降圧薬の選択を行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行われた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pharmacogenomicsを重視して研究を行ってきた．本稿では，その成果を中心に，高血圧のテーラーメイド診療の確立の可能性について述べる．

降圧薬のpharmacogenomics

一般に降圧薬を含む多くの薬剤では，遺伝因子を含め種々の因子は，それぞれ小さいが累積することにより薬効に影響が及ぶ正規分布型の反応を示すため，影響を及ぼす遺伝子変異や多型を同定するのは容易ではないとされる．これまでに報告された降圧薬の効果に関連する遺伝子多型の多くは，CYPなどの薬物代謝酵素関連の遺伝子からアプローチする，pharmacokineticsよりも，受容体や薬物関連のペプチドやホルモンなどの遺伝子を検討する，pharmacodynamicsの観点より検討されてきた．

その理由として，Turnerは以下のように推察している[5]．現在，多型により多様性のある薬物代謝酵素で代謝される降圧薬（hydralazine，methyldopa，β遮断薬など）は，多用されなくなっている．また効果や副作用の発現に個人差が大きい降圧薬は，市場に生き残れない．さらに実験的にヒトの薬物代謝を評価する方法の確立に伴い，一つの遺伝子多型により多様性を示す酵素でもっぱら代謝される降圧薬の出現の可能性は非常に低い，などの理由により，pharmacodynamicな機序が，今現在広く使用されている降圧薬ではその効果の個人差に強く関与しているであろうと考えられているからである．また，そのアプローチ法はほとんどが候補遺伝子であるが，最近，GWASを用いた解析の成果も報告されている[6]．以下に，主要降圧薬の感受性遺伝子研究の現況を述べる．

1. サイアザイド利尿薬（TD）

サイアザイド利尿薬（TD）はアメリカの高血圧治療ガイドラインで唯一の第一選択薬とされており，また，遺伝的素因の影響力の強い食塩感受性との関連が強いため，関連遺伝子がもっともよく検討されている．代表的なものは，G蛋白β3サブユニット遺伝子（GNB3）C825T多型[7]，α-Adducin遺伝子（ADD1）Gly460Trp多型[8]の二つである．GNB3のC825T多型はβ3-shortを生じ，高血圧の原因遺伝子変異の一つとも考えられている．GNB3は12p13領域に位置し，その第10エクソン上にC825Tは存在する．C825Tはサイレントな変異であるが，スプライシングの異常を生じその結果，第8，9エクソンの一部に対応する41残基を欠失するβ3-shortを産生する確率を上げると推定されている．

β3-shortで欠失する部分は，G蛋白αサブユニット（Gα）との相互作用に重要な場所に位置しているため，受容体によるGαの活性化を促進すると考えられている．このGNB3のC825T多型が低レニン活性と関連することが報告されたため，TDの効果にも影響することが予測され，197人のアフリカ系アメリカ人と190人のコーカソイドで検討された結果，CC, CT, TTの順に有意にTDによる降圧効果が良好であった[7]．

Adducinは，細胞膜骨格蛋白で$\alpha\beta$のヘテロ二量体を形成する．Milan高血圧ラットの解析でαとβ adducin遺伝子のミスセンス変異が腎臓でのNa再吸収亢進に関与し，高血圧を呈することから，ヒトにおいてもADD1の遺伝子多型と高血

圧との関連が検討され，Gly460Trp 多型で有意な関係が認められた．日本人でも低レニン性高血圧には有意な関連を示すため，食塩感受性に影響を及ぼしているものと考えられるが，高血圧・低レニンの多い Trp460 アレルの保有者では TD への反応性が良好であった[8]．これらの成績はすべて欧米からのもので，日本人である程度大規模な TD の効果に関連する遺伝子変異・多型の報告はなされていなかった．

われわれは，76人の新規 TD 服用患者の降圧効果から，感受性遺伝子多型の同定を後ろ向きの解析手法にて試みた[9]．外来受診中の高血圧患者で新規に TD が処方された患者を対象に，服用開始前後 3 回の外来血圧を平均し，平均血圧で 5 mmHg 以上の降圧を認めた群を反応群（responder：R 群），それ未満もしくは投与後血圧が上昇した群を非反応群（non responder：NR 群）と定義した．検討した遺伝子多型は GNB3 C825T, ADD1 Gly460Trp, RAS や交感神経系（SNS）関連遺伝子に加え，サイアザイド感受性 Na-Cl 共輸送体遺伝子（TSC），TD 感受性の Gordon 症候群の原因遺伝子である WNK1, WNK4，ミネラルコルチコイド受容体遺伝子（MLR）などをダイレクト・シークエンスにより同定した SNPs，合計17遺伝子，48多型をタイピングした．その結果，TSC C1784T と ADRB3 T727C（Trp64 Arg）の 2SNPs が R・NR 群における頻度の差，ならびに実際の降圧度においても多型間に有意な差を認めた．しかしながら，前述した GNB3 C825T, ADD1 Gly460Trp では有意な相関を認めなかった．これら GNB3 や ADD1 の多型は日本人高血圧への関与でも否定的な報告がされており，人種差を反映している可能性がある．

最近のゲノム解析研究は候補遺伝子アプローチではなく，GWAS が主流となっている．降圧薬関連では後述するわれわれの研究の他に，TD に関しては昨年，米国から報告があった[6]．この研究では約300人の黒人高血圧患者において，hydrochlorothiazide 25 mg を 1 ヵ月間投与した前後の拡張期血圧値を用い，効果良好，不良を示したそれぞれ97名を解析に用いている．遺伝子型の決定は10万 SNP を網羅した DNA チップを用い，GWAS を行ったところ，染色体12番に多重検定補正後も有意性を示す SNP 群（LYZ, YEATS4, FRG2）が存在した．LYZ, FRG2 の SNPs は，白人のサンプルを用いて関連解析をしたところ有意な関連を示し，再現性がとれたと報告された．現在のところ，LYZ, FRG2 と TD の作用との関係は不明であるが，GWAS が降圧薬の pharmacogenomics に応用可能であることを示した研究として意義がある．

2. アンジオテンシンⅡ受容体拮抗薬（ARB）

スウェーデンの研究グループは，アンジオテンシンⅡ受容体拮抗薬（ARB）（irbesartan）の降圧ならびに心肥大抑制効果に対する遺伝子多型の関与を，SILVHIA（Swedish Irbesartan Left Ventricular Hypertrophy Investigation vs. Atenolol）研究として勢力的に検討している[10]．この研究では irbesartan と atenolol をそれぞれ50名程度の高血圧患者に12週間，単剤投与するといったプロトコールで，それぞれの薬剤の降圧効果，心肥大退縮作用を検討したものである．

irbesartan は軽・中等症高血圧患者の40～50％で有効な降圧効果をもつ薬とされているが，ACE I/D 多型のII型を示す患者の89％に拡張期血圧（DBP）で 10 mmHg 以上の降圧を認めた．一方，DD 型では24％しか DBP＞10 mmHg 以上の降圧を示した患者はいなかった[10]．同様にアルドステロン合成酵素遺伝子 C-344T も，irbesartan の降圧効果に有意な関連性を示していた．他の RAS 関連の遺伝子多型では降圧効果に有意性を認めたものはなかったが，アンジオテンシノーゲン遺伝子 T174M, M235T ならびにアンジオテンシン 1 型受容体遺伝子 A1166C は，irbesartan の心肥大退縮作用に有意性を認めた．

またこの研究グループは，マイクロアレイを用いた独自のタイピング法（microarray based DNA polymerase assisted minisequencing single nucleotide primer extension assay with fluores-

cence detection）を開発し，SILVHIA研究においてそれぞれの薬剤の降圧効果[11]や心肥大退縮作用[12]に関与するSNPを，RASやSNS，血管作動性物質，脂質代謝などに関わる25遺伝子，74SNPsで検討して，複数の薬剤感受性遺伝子多型を同定している．現在のところ，この方法はpharmacogenomicsによるテーラーメイド医療に応用されているとの報告はないが，このようなカスタムメイドチップが臨床応用に用いられることになると予測される．

われわれも，ARBの効果に影響するSNPの検討を行っている．CYP2C9が，losartanやvalsartanの薬物代謝に関わることが知られているが，CYP2C9遺伝子の翻訳領域をリシークエンスし，新規のミスセンス変異を同定している[13]．また，CYP2C9に機能的変化をもたらす変異CYP2C9*30（Ala447Thr）を有する患者ではlosartanが効かない可能性があり，1.4%程度と頻度は少ないがテーラーメイド医療を行う際には考慮を要すると思われる．

3．ジヒドロピリジン系カルシウム拮抗薬（CCB）

ジヒドロピリジン系カルシウム拮抗薬（CCB）の降圧効果に明らかに関与する遺伝子多型は，ほとんど報告がない．とくにジヒドロピリジン系CCBは，わが国のみならず国際的にも使用頻度が高い薬剤であるために，感受性遺伝子が存在するならその同定における意義は大変大きいと考えられる．

われわれはL型カルシウムチャンネル遺伝子の多型に注目し，新規にCCBが処方された161名の本態性高血圧患者を対象として，TDのときと同様の降圧基準にて反応（R）群，非反応（NR）群に分け，L型カルシウムチャネル・サブユニット遺伝子（α1C: CACNA1C, α1D: CACNA1D）を選択し，アレル頻度の高い計16多型について解析した．この結果，CACNA1Cのイントロン13, G/A多型，CACNA1Dのイントロン3 G/AならびにC/Tの2多型が，R, NR群の頻度ならびにCCB投与後の血圧値にアレル間で有意な差を認めたため，CCBの応答性に影響を与えているものと考えている[14]．CACNA1SのSNPは欧米のグループも同様の報告をしており，有望な関

図1　GEANE研究の研究体制とプロトコール

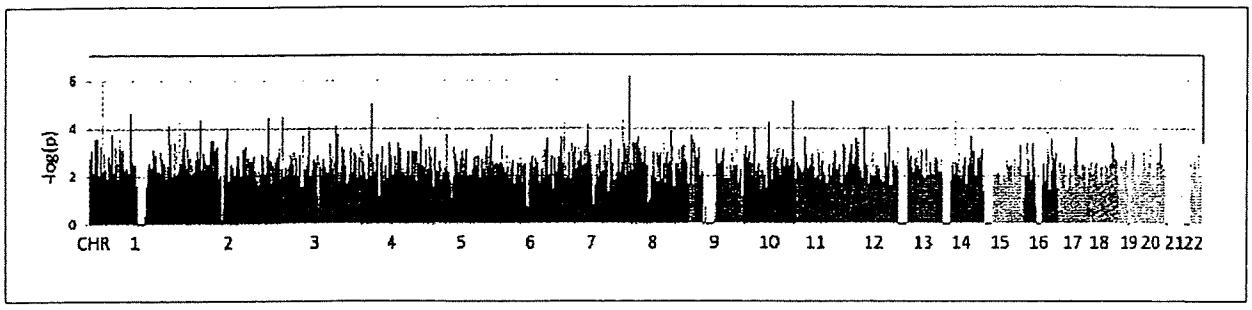

図2　サイアザイド利尿薬関連 SNP
indapamide 投与後の血圧値を投薬前の血圧値で補正後，レスポンダー・ノンレスポンダーを判定し，GWAS を行った．染色体7番に $p<10^{-6}$ を超えるもっとも強い関連 SNP を認めた．

連遺伝子と考えられる．

GEANE 研究

降圧薬関連遺伝子多型は複数存在することが予測され，明らかな薬剤応答性・感受性遺伝子の同定のためには，多数例の無治療高血圧患者に前向きに降圧薬を投与し，正確に降圧の程度を把握し，数多くの薬物代謝酵素や薬理作用機序関連の遺伝子多型との相関を検討する必要がある．これまで世界でもこのような研究はなかったが，国立循環器病センターでは全国の大学・医療センター計24施設とともに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 研究：Gene Evaluation for ANtihypertensive drug Effect）を施行した[15]（図1）．

GEANE 研究では同一患者にサイアザイド類利尿薬（indapamide），ジヒドロピリジン系カルシウム拮抗薬（amlodipine），アンジオテンシンⅡ受容体拮抗薬（valsartan）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 研究ではこれら3剤の降圧効果関連 SNP のみならず，TD 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 の効果関連でもっとも強い関連性を示す SNP は，染色体7番にあり，前述した米国からの報告にあった12番の領域には強い関連性は認めなかった．その他，CCB, 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 研究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．

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前向き試験により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．われわれは GEANE2 研究を計画しており，遺伝子多型による降圧薬選択の有用性を前向きに多施設共同研究にて検証する予定である．今後は遺伝子多型診断を考慮した，新しい高血圧診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　本稿中の研究成果は，独立行政法人医薬品医療機器総合機構の「保健医療分野における基礎研究推進事業」より助成金を得たミレニアムゲノムプロジェクト（高血圧等循環器疾患における遺伝子解析，創薬推進事業，研究代表者：国立循環器病センター病院長　友池仁暢）ならびに医薬基盤研究所に

よる研究課題（高血圧等循環器疾患のゲノム情報多元的意義付けと画期的診断・治療法の開発，研究代表者：国立循環器病センター研究所部長　森崎隆幸）によるものである．また降圧薬関連遺伝子多型の前向き臨床試験は，2005～2008年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病院部長　河野雄平）のサポートを受けている．

文　献

1) Dominiczak AF, Negrin DC, Clark JS et al: Genes and hypertension. Hypertension 2000; **35**: 164–172
2) Arnett DK, Baird AE, Barkley RA et al: Relevance of genetics and genomics for prevention and treatment of cardiovascular disease: a scientific statement from the American Heart Association Council on Epidemiology and Prevention, the Stroke Council, and the Functional Genomics and Translational Biology Interdisciplinary Working Group. Circulation 2007; **115**: 2878–2901
3) Kamide K, Takiuchi S, Miyata T et al: Single nucleotide polymorphisms analysis of hypertension relating to the effect of antihypertensive drugs: Millennium Genome Project at NCVC. Jpn Heart J 2004; **45** (Suppl): S69–S93
4) The Wellcome Trust Case Control Consortium: Genome-wide association study of 14,000 cases of seven common diseases and 3,000 shared control. Nature 2007; **447**: 661
5) Turner ST, Schwartz GL, Chapman AB et al: Antihypertensive pharmacogenetics: getting the right drug into the right patient. J Hypertens 2001; **19**: 1–11
6) Turner ST, Bally KR, Fridley BL et al: Genomic association analysis suggests chromosome 12 locus influencing antihypertensive response to thiazide diuretic. Hypertension 2008; **52**: 359–365
7) Turner ST, Schwartz GL, Chapman AB et al: C825T polymorphism of the G protein beta(3)-subunit and antihypertensive response to a thiazide diuretic. Hypertension 2001; **37**: 739–743
8) Cusi D, Barlassina C, Azzani T et al: Polymorphisms of alpha-adducin and salt sensitivity in patients with essential hypertension. Lancet 1997; **349**: 1353–1357
9) Matayoshi T, Kamide K, Takiuchi S et al: 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. Hypertens Res 2004; **27**: 821–833
10) Kurland L, Melhus H, Karlsson J et al: Angiotensin converting enzyme gene polymorphism predicts blood pressure response to angiotensin II receptor type 1 antagonist treatment in hypertensive patients. J Hypertens 2001; **19**: 1783–1787
11) Liljedahl U, Karlsson J, Melhus H et al: A microarray minisequencing system for pharmacogenetic profiling of antihypertensive drug response. Pharmacogenetics 2003; **13**: 7–17
12) Liljedahl U, Kahan T, Malmqvist K et al: Single nucleotide polymorphisms predict the change in left ventricular mass in response to antihypertensive treatment. J Hypertens 2004; **22**: 2273–2275
13) Yin T, Maekawa K, Kamide K et al: Genetic variations of CYP2C9 in 724 Japanese individuals and their impact on the antihypertensive effects of losartan. Hypertens Res 2008; **31**: 1549–1557
14) Kamide K, Yang J, Matayoshi T et al: Genetic polymorphisms of L-type calcium channel α1C and α1D subunit genes are associated with sensitivity to the antihypertensive effects of L-type dihydropyridine calcium-channel blockers. Circ J, 2009 (in press)
15) 神出　計，花田裕典，宮田敏行ほか：第31回日本高血圧学会総会抄録集，2008

Circ J 2009; **73:** 732–740

Genetic Polymorphisms of L-Type Calcium Channel α1C and α1D Subunit Genes are Associated With Sensitivity to the Antihypertensive Effects of L-Type Dihydropyridine Calcium-Channel Blockers

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Background: The response of blood pressure (BP) to L-type dihydropyridine calcium-channel blockers (dCCBs) differs among individuals.

Methods and Results: A pharmacogenomic analysis was undertaken in 161 patients with essential hypertension who were treated with dCCBs to study whether genetic polymorphisms of the calcium channel α1C and α1D subunit genes, *CACNA1C* and *CACNA1D*, are associated with the antihypertensive effects of dCCBs. Responders were defined as those in whom systolic BP (SBP) was lowered by more than 20 mmHg or diastolic BP (DBP) was lowered by more than 10 mmHg after treatment with dCCBs. Eleven sequence-proven polymorphisms of *CACNA1C* and 5 common polymorphisms of *CACNA1D* chosen from a public database were subjected to genotypic analysis. The comparison of polymorphism prevalence between responders and nonresponders showed significant differences in *CACNA1D* rs312481G>A and rs3774426C>T, and in *CACNA1C* 527974G>A. There were significant differences in SBP or DBP between alleles in these single nucleotide polymorphisms (SNPs). A much more significant reduction in BP was observed for the combined presence of these SNPs.

Conclusions: Three SNPs in *CACNA1D* or *CACNA1C* are genetic polymorphisms conferring sensitivity to the antihypertensive effects of L-type dCCBs in patients with hypertension. The BP reduction by L-type dCCBs might be predicted by evaluating these polymorphisms. (*Circ J* 2009; **73:** 732–740)

Key Words: Essential hypertension; Genetic polymorphism; L-type calcium channel gene; L-type dihydropyridine channel blockers

Calcium-channel blockers (CCBs), especially those of the L-type dihydropyridine (DHP) subclass, are widely used to treat hypertension because they are better able to lower blood pressure (BP) than are other types of antihypertensive agents! The DHP CCBs (dCCBs) complement the BP-lowering ability in both salt-sensitive and salt-resistant forms of hypertension (HT), and elderly patients generally respond well to dCCBs[2] Recently, a number of trials, including VALUE[3] ALLHAT[4] ASCOT[5] Syst-Eur[6] and STONE[7] and most correctly performed meta-analyses have demonstrated that dCCBs effectively reduce the incidence of stroke events in older patients with HT[6,7] and could be the preferred agents for treating HT in patients with ischemia heart diseases because of their vasodilatory effects on the coronary arteries[3,8,9] Moreover, dCCBs demonstrate additive effects on BP reduction by most other kinds of antihypertensive agents, especially angiotensin-I-converting enzyme inhibitors, angiotensin-II-receptor blockers, β-blockers, and thiazide diuretics, with few side-effects[2,10] Because of these advantages of dCCBs, several groups that establish international guidelines have recently endorsed them as an initial therapy option in patients with essential HT (EHT), and as an important component of most multidrug regimens for BP control according to a Japanese guideline (JSH2004)![11] However, the response of BP to dCCBs differs among individuals, so, to lower BP more effectively, determining an individual's sensitivity to a dCCB before prescribing it would be useful.

Recent studies indicate that the heterogeneity of a patient's responses to antihypertensive treatment is, at least in part, genetically determined![12] This finding underscores the role of pharmacogenetic research to identify either functional genetic variations or variations inherited in linkage disequilibrium (LD) with these variations as markers to enable more individualized evaluation and selection of agents for treating HT in each drug class![13]

In Japan, more than a dozen dCCBs, particularly DHP derivatives, are available for clinical use. These DHP derivatives bind to receptors on the α1 subunits of DHP-sensitive voltage-gated (L-type) calcium channels to exert their antihypertensive effects and are believed to play a central role in the excitation–contraction coupling for cardiac and

(Received August 11, 2008; revised manuscript received October 27, 2008; accepted November 12, 2008; released online February 17, 2009)

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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![4] 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![5] 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![6] In most cases, both channel types are found in the same cells, with α1C usually being the predominant isoform![7] 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)![8] and that α1D might control physiological processes, such as diastolic depolarization in sinoatrial node cells and neurotransmitter release and neuronal excitability![9] 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![10] 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%),

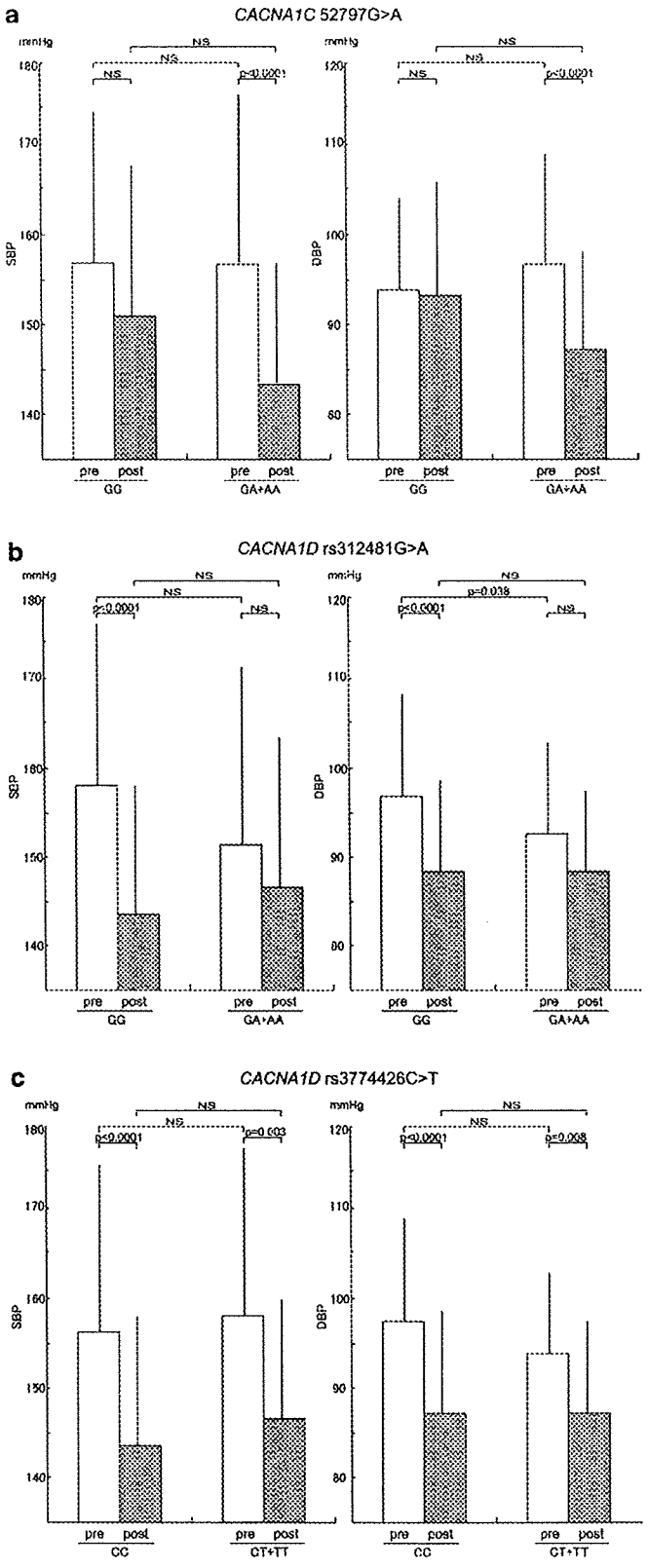

Figure. (a) Comparison of blood pressure (BP) between pre- and post-administration of CCBs in each genotype of dominant model in patients with *CACNA1C* 527974G>A. (b) Comparison of BP between pre- and post-administration of CCBs in each genotype of recessive model in patients with *CACNA1D* rs312481G>A. (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 20mmHg or a DBP reduction greater than 10mmHg 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 1,000bp 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 >10mmHg, 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 >20mmHg, 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. 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.

Acknowledgments

This study was supported by the Program for Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation (NIBIO) of Japan and a Research Grant from the Ministry of Health, Labor and Welfare of Japan.

References

1. Mori H, Ukai H, Yamamoto H, Saitou S, Hirao K, Yamauchi M, et al. Current status of antihypertensive prescription and associated blood pressure control in Japan. *Hypertens Res* 2006; **29:** 143–151.
2. Sica DA. Pharmacotherapy review: Calcium channel blockers. *J Clin Hypertens (Greenwich)* 2006; **8:** 53–56.
3. Julius S, Kjeldsen SE, Weber M, Brunner HR, Ekman S, Hansson L, et al. VALUE trial group. Outcomes in hypertensive patients at high cardiovascular risk treated with regimens based on valsartan or amlodipine: The VALUE randomised trial. *Lancet* 2004; **363:** 2022–2031.
4. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 2002; **288:** 2981–2997.
5. Dahlof B, Sever PS, Poulter NR, Wedel H, Beevers DG, Caulfield M, et al, ASCOT Investigators. Prevention of cardiovascular events with an antihypertensive regimen of amlodipine adding perindopril as required versus atenolol adding bendroflumethiazide as required in the Anglo-Scandinavian Cardiac Outcomes Trial–Blood Pressure Lowering Arm (ASCOT-BPLA): A multicenter randomized controlled trial. *Lancet* 2005; **366:** 895–906.
6. Staessen JA, Fagard R, Thijs L, Celis H, Arabidze GG, Birkenhäger WH, et al. Randomised double blind comparison of placebo and active treatment for older patients with isolated systolic hypertension. *Lancet* 1997; **350:** 757–764.
7. Gong L, Zhang W, Zhu Y, Zhu J, Kong D, Pagé V, et al. Shanghai trial of nifedipine in the elderly (STONE). *J Hypertens* 1996; **14:** 1237–1245.
8. Nissen SE, Tuzcu EM, Libby P, Thompson PD, Ghali M, Garza D, et al. CAMELOT Investigators. Effect of antihypertensive agents on cardiovascular events in patients with coronary disease and normal blood pressure: The CAMELOT study: A randomized controlled trial. *JAMA* 2004; **294:** 2217–2226.
9. Yui Y, Hirayama A, Nonogi H, Kimura K, Kodama K, Hosoda S, et al. Unstable angina and non-ST elevation acute coronary syndrome: Epidemiology and current management in Japan (Japan Multicenter Investigation for Cardiovascular Disease-D (JMIC-D) Committee). *Circ J* 2007; **71:** 1335–1347.
10. Takase B, Takeishi Y, Hirai T, Lee JD, Uzui H, Senda S, et al. Comparative effects of amlodipine monotherapy and combination therapy with betaxolol on cardiac autonomic nervous activity and health-related quality of life in patients with poorly controlled hypertension. *Circ J* 2008; **72:** 764–769.
11. Japanese Society of Hypertension. Guidelines for the management of hypertension (JSH2004). *Hypertens Res* 2006; 29(Suppl): S1–S105.
12. Kurland L, Liljedahl U, Lind L. Hypertension and SNP genotyping in antihypertensive treatment. *Cardiovasc Toxicol* 2005; **5:** 133–142.
13. Doris PA. Hypertension genetics, single nucleotide polymorphisms, and the common disease: Common variant hypothesis. *Hypertension* 2002; **39:** 323–331.
14. Snutch TP, Reiner PB. Ca^{2+} channels: Diversity of form and function. *Curr Opin Neurobiol* 1992; **2:** 247–253.
15. Ertel EA, Campbell KP, Harpold MM, Hofmann F, Mori Y, Perez-Reyes E, et al. Nomenclature of voltage-gated calcium channels. *Neuron* 2000; **25:** 533–535.
16. Lipscombe D, Helton TD, Xu W. L-type calcium channels: The low down. *J Neurophysiol* 2004; **92:** 2633–2641.
17. Hell JW, Westenbroek RE, Warner C, Ahlijanian MK, Prystay W, Gilbert MM, et al. Identification and differential subcellular localization of the neuronal class C and class D L-type calcium channel alpha 1 subunits. *J Cell Biol* 1993; **123:** 949–962.
18. Sinnegger-Brauns MJ, Hetzenauer A, Huber IG, Renström E, Wietzorrek G, Berjukov S, et al. Isoform-specific regulation of mood behavior and pancreatic beta cell and cardiovascular function by L-type Ca^{2+} channels. *J Clin Invest* 2004; **113:** 1430–1439.
19. Koschak A, Reimer D, Huber I, Grabner M, Glossmann H, Engel J, et al. alpha 1D (Cav1.3) subunits can form l-type Ca^{2+} channels activating at negative voltages. *J Biol Chem* 2001; **276:** 22100–22106.
20. Osborn JW, Jacob F, Guzman P. A neural set point for the long-term control of arterial pressure: Beyond the arterial baroreceptor reflex. *Am J Physiol Regul Integr Comp Physiol* 2005; **288:** R846–R855.
21. Okuda T, Fujioka Y, Kamide K, Kawano Y, Goto Y, Yoshimasa Y, et al. Verification of 525 coding SNPs in 179 hypertension candidate genes in the Japanese population: Identification of 159 SNPs in 93 genes. *J Hum Genet* 2002; **47:** 387–394.
22. Seisenberger C, Specht V, Welling A, Platzer J, Pfeifer A, Kühbandner S, et al. Functional embryonic cardiomyocytes after disruption of the L-type alpha1C (Cav1.2) calcium channel gene in the mouse. *J Biol Chem* 2000; **275:** 39193–39199.
23. Moosmang S, Schulla V, Welling A, Feil R, Feil S, Wegener JW, et al. Dominant role of smooth muscle L-type calcium channel Cav1.2 for blood pressure regulation. *EMBO J* 2003; **22:** 6027–6034.
24. Grossman E, Messerli FH. Calcium antagonists. *Prog Cardiovasc Dis* 2004; **47:** 34–57.
25. Bremer T, Man A, Kask K, Diamond C. CACNA1C polymorphisms are associated with the efficacy of calcium channel blockers in the treatment of hypertension. *Pharmacogenomics* 2006; **7:** 271–279.
26. Wang D, Papp AC, Binkley PF, Johnson JA, Sadée W. Highly variable mRNA expression and splicing of L-type voltage-dependent calcium channel alpha subunit 1C in human heart tissues. *Pharmacogenet Genomics* 2006; **16:** 735–745.
27. Tang ZZ, Liang MC, Lu S, Yu D, Yu CY, Yue DT, et al. Transcript scanning reveals novel and extensive splice variations in human l-type voltage-gated calcium channel, Cav1.2 alpha1 subunit. *J Biol Chem* 2004; **279:** 44335–44343.
28. Soldatov NM, Oz M, O'Brien KA, Abernethy DR, Morad M. Molecular determinants of L-type Ca^{2+} channel inactivation: Segment exchange analysis of the carboxyl-terminal cytoplasmic motif encoded by exons 40–42 of the human alpha1C subunit gene. *J Biol Chem* 1998; **273:** 957–963.
29. Liao P, Yu D, Lu S, Tang Z, Liang MC, Zeng S, et al. Smooth muscle-selective alternatively spliced exon generates functional variation in Cav1.2 calcium channels. *J Biol Chem* 2004; **279:** 50329–50335.
30. Lacinova L, Klugbauer N, Hofmann F. State- and isoform-dependent interaction of isradipine with the alpha1C L-type calcium channel. *Pflugers Arch* 2000; **440:** 50–60.
31. Nissim-Rafinia M, Kerem B. Splicing regulation as a potential genetic modifier. *Trends Genet* 2002; **18:**123–127.

32. Xu W. Lipscombe D. Neuronal Ca(V)1.3alpha(1) L-type channels activate at relatively hyperpolarized membrane potentials and are incompletely inhibited by dihydropyridines. *J Neurosci* 2001; **21:** 5944–5951.
33. Bean BP. Nitrendipine block of cardiac calcium channels: High-affinity binding to the inactivated state. *Proc Natl Acad Sci USA* 1984; **81:** 6388–6392.
34. Berjukow S, Hering S. Voltage-dependent acceleration of Ca (v)1.2 channel current decay by (+)- and (–)-isradipine. *Br J Pharmacol* 2001; **133:** 959–966.
35. Safa P, Boulter J, Hales TG. Functional properties of Cav1.3 (alpha1D) L-type Ca^{2+} channel splice variants expressed by rat brain and neuroendocrine GH3 cells. *J Biol Chem* 2001; **276:** 38727–38737.
36. Takimoto K, Li D, Nerbonne JM, Levitan ES. Distribution, splicing and glucocorticoid-induced expression of cardiac alpha 1C and alpha 1D voltage-gated Ca^{2+} channel mRNAs. *J Mol Cell Cardiol* 1997; **29:** 3035–3042.
37. Lohmueller KE, Pearce CL, Pike M, Lander ES, Hirschhorn JN. Meta-analysis of genetic association studies supports a contribution of common variants to susceptibility to common disease. *Nat Genet* 2003; **33:** 177–182.

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