Table 2. Meta-Analysis of ATP2B1 SNPs With BP Traits

		Millennium GPJ			Global BPgen			CHARGE*			Pooled	
SNP	Coded Allele	n (Frequency)	Coefficient (SE), mm Hg	*P*	n (Frequency)	Coefficient (SE), mm Hg	*P*	n (Frequency)	Coefficient (SE), mm Hg	*P*	Coefficient (95% CI), mm Hg	*P*
SBP												
rs1401982	G	13 944	−1.22	1.8×10^{-7}	33 885	−0.30	0.022				−0.52	3.9×10^{-6}
		(0.376)	(0.23)		(0.385)	(0.13)					(−0.74 to −0.30)	
rs2681472	G	14 032	−1.33	1.2×10^{-8}	33 803	−0.62	5.2×10^{-4}	0.17	−1.29	3.5×10^{-11}	−1.03	9.9×10^{-20}
		(0.373)	(0.23)		(0.158)	(0.18)			(0.19)		(−1.26 to −0.81)	
rs11105364	G	14 013	−1.34	8.9×10^{-9}	33 877	−0.60	7.4×10^{-4}	0.17	−1.30	4.8×10^{-11}	−1.03	1.2×10^{-19}
		(0.364)	(0.23)		(0.179)	(0.18)			(0.19)		(−1.25 to −0.81)	
rs11105378	T	13 948	−1.33	1.5×10^{-8}	33 171	−0.59	0.001	0.16	−1.31	9.1×10^{-11}	−1.02	1.4×10^{-18}
		(0.360)	(0.23)		(0.158)	(0.18)			(0.20)		(−1.24 to −0.79)	
DBP												
rs1401982	G	13 944	−0.72	2.0×10^{-7}	33 898	−0.18	0.041				−0.34	8.1×10^{-6}
		(0.376)	(0.14)		(0.392)	(0.09)					(−0.49 to −0.19)	
rs2681472	G	14 032	−0.65	2.7×10^{-6}	33 829	−0.35	0.003	0.17	−0.64	3.7×10^{-8}	−0.54	9.7×10^{-15}
		(0.373)	(0.14)		(0.157)	(0.12)			(0.11)		(−0.68 to −0.41)	
rs11105364	G	14 013	−0.70	4.5×10^{-7}	33 898	−0.34	0.004	0.17	−0.63	1.2×10^{-7}	−0.54	7.5×10^{-14}
		(0.364)	(0.14)		(0.158)	(0.12)			(0.12)		(−0.68 to −0.40)	
rs11105378	T	13 948	−0.70	5.4×10^{-7}	33 183	−0.33	0.005	0.16	−0.62	3.1×10^{-7}	−0.54	1.6×10^{-13}
		(0.360)	(0.14)		(0.158)	(0.12)			(0.12)		(−0.68 to −0.39)	

Coefficients and SE for SBP and DBP were calculated under the additive model using multiple regression analysis adjusted for age, age^2, sex, and BMI. In both Millennium GPJ and Global BPgen, adjustment for treatment with antihypertensive medication was achieved by adding fixed constants to measured values (+15 mm Hg for SBP and +10 mm Hg for DBP).[2] In the Japanese Millennium GPJ and also for some cohorts within Global BPgen, cohort variables were also adjusted to avoid residual population stratification.

*Results of the CHARGE Study were obtained from the published article.[3]

ATP2B1. To further validate and get more precise effect size estimates in Japanese, for this analysis, hypertensive cases were defined as individuals with treatment with antihypertensive medication, SBP ≥140 mm Hg, or DBP ≥90 mm Hg. The ORs for the 4 SNPs were all extremely similar (ranging from 1.19 to 1.21 under the additive model adjusted for age, age^2, sex, BMI, and cohort variables; see Table S7). These associations were replicated in the Global BPgen subjects of European descent; the pooled analysis demonstrated increased significance (rs1105378: OR: 1.17 [95% CI: 1.11 to 1.23]; $P=7.0\times10^{-10}$), as expected for a larger total sample size (n=28 866; Table S7).

We next evaluated the effect of the most associated SNP, rs11105378, on BP levels in the Millennium GPJ cohort (Table 2). We adjusted for several covariates that are associated with BP phenotypes: age ($r=0.362$; $P<0.001$ for SBP), BMI ($r=0.275$; $P<0.001$), and sex (male: 131.7±18.2; female: 128.6±20.8 mm Hg; $P<0.001$). In multiple regression analysis for BP levels, including also cohort indicator variables as covariates, the results for a 2-degree-of-freedom test with the TT genotype as a reference identified both the TC genotype (coefficient=+1.66 mm Hg; $P=2.2\times10^{-4}$) and CC genotype (+2.47 mm Hg; $P=4.9\times10^{-8}$) as independent determinants for SBP after adjustment. The TC (+0.91 mm Hg; $P=8.0\times10^{-4}$) and CC genotypes (+1.32 mm Hg; $P=1.8\times10^{-6}$) were also independently associated with DBP levels. We depict the covariate adjusted mean BP levels by rs11105378 genotype in Figure S3. Results of each cohort separately are summarized in Table S8. We next performed a meta-analysis of data from the Millennium GPJ and 2 large epidemiological studies (Global BPgen and CHARGE; Table 2). Results show the per-allele differences in SBP and DBP to be ≈1.0 and 0.5 mm Hg, respectively.

Genotype-Specific Differences in Ex Vivo Expression of ATP2B1 mRNA

Differences in *ATP2B1* mRNA expression in umbilical artery smooth muscle cells among rs11105738 genotype are shown in Figure 1. Assuming a recessive genetic model, cells homozygous for T allele showed significantly higher levels of

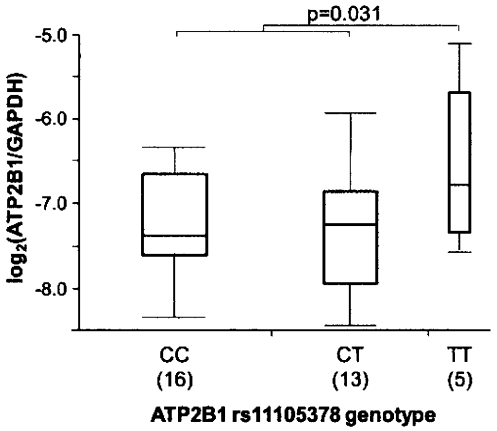

Figure 1. Ex vivo expression analysis of *ATP2B1* mRNA. Graphs depict the log^2 relative expression levels of the *ATP2B1* mRNA in umbilical artery smooth muscle cells obtained by normalizing to GAPDH. Genotype of *ATP2B1* rs11105378 of each sample was analyzed by direct sequencing using isolated genomic DNA from umbilical artery smooth muscle cells.

Table 3. Meta-Analysis of SNPs With BP Traits

SNP	Coded Allele	Millennium GPJ n (Frequency)	Millennium GPJ Coefficient (SE), mm Hg	Millennium GPJ P	Global BPgen n (Frequency)	Global BPgen Coefficient (SE), mm Hg	Global BPgen P	Pooled Coefficient (95% CI), mm Hg	Pooled P
Systolic BP									
FGF5	T	13 826	1.33	1.6×10^{-8}	30 850	0.62	1.6×10^{-6}	0.81	1.1×10^{-11}
rs1458038		(0.343)	(0.23)		(0.275)	(0.14)		(0.58 to 1.05)	
CYP17A1	A	14 007	0.89	2.3×10^{-4}	33 735	0.94	1.0×10^{-5}	0.92	6.2×10^{-9}
rs1004467		(0.680)	(0.24)		(0.901)	(0.21)		(0.61 to 1.23)	
CSK	C	13 920	0.77	0.007	34 126	0.62	2.4×10^{-6}	0.65	4.2×10^{-8}
rs1378942		(0.803)	(0.28)		(0.36)	(0.13)		(0.42 to 0.88)	
PLCD3	T	14 003	0.11	0.703	32 120	0.68	3.9×10^{-6}	0.57	2.5×10^{-5}
rs12946454		(0.831)	(0.30)		(0.28)	(0.15)		(0.30 to 0.83)	
PLEKHA7	T	14 030	0.11	0.687	33 706	0.52	2.6×10^{-4}	0.44	4.7×10^{-4}
rs381815		(0.199)	(0.28)		(0.26)	(0.14)		(0.19 to 0.68)	
CSK-ULK3	A	14 014	0.68	0.017	33 308	0.47	2.4×10^{-4}	0.51	1.7×10^{-5}
rs6495122		(0.812)	(0.28)		(0.45)	(0.13)		(0.28 to 0.74)	
ULK4	A	13 976	−0.67	0.059	32 034	0.17	0.297	0.01	0.950
rs9815354		(0.116)	(0.35)		(0.18)	(0.17)		(−0.29 to 0.31)	
DBP									
FGF5	T	13 826	0.73	1.8×10^{-7}	30 850	0.55	1.5×10^{-8}	0.61	6.1×10^{-14}
rs1458038		(0.343)	(0.14)		(0.275)	(0.10)		(0.45 to 0.77)	
CYP17A1	A	14 007	0.29	0.047	33 735	0.40	5.4×10^{-3}	0.35	4.9×10^{-4}
rs1004467		(0.680)	(0.14)		(0.901)	(0.14)		(0.15 to 0.54)	
CSK	C	13 920	0.41	0.015	34 126	0.48	5.9×10^{-8}	0.46	5.2×10^{-9}
rs1378942		(0.803)	(0.17)		(0.36)	(0.09)		(0.31 to 0.62)	
PLCD3	T	14 003	0.14	0.426	32 120	0.34	5.7×10^{-4}	0.30	1.9×10^{-4}
rs12946454		(0.831)	(0.18)		(0.28)	(0.09)		(0.14 to 0.46)	
PLEKHA7	T	14 030	0.13	0.437	33 706	0.23	0.014	0.20	0.018
rs381815		(0.199)	(0.17)		(0.26)	(0.10)		(0.04 to 0.37)	
CSK-ULK3	A	14 014	0.38	0.027	33 308	0.35	4.2×10^{-5}	0.36	7.4×10^{-6}
rs6495122		(0.812)	(0.17)		(0.45)	(0.09)		(0.20 to 0.51)	
ULK4	A	13 976	0.21	0.325	32 034	0.40	2.9×10^{-4}	0.36	2.3×10^{-4}
rs9815354		(0.116)	(0.21)		(0.18)	(0.11)		(0.17 to 0.55)	

ATP2B1 mRNA as compared with cells carrying 1 or 2 C alleles ($P=0.031$; see Figure 1). Under an additive genetic model, the overall P value was marginally significant ($P=0.091$).

Replication Analysis of European GWAS-Derived Susceptible SNPs in Japanese

We next conducted a replication analysis in the Millennium GPJ, in which we tested associated SNPs identified in recent large-scale European GWAS by the Global BPgen[2] and the CHARGE consortia.[3] From the 7 most promising SNPs of which the minor allele frequency in Japanese was >0.10 based on the HapMap database, 4 SNPs, namely, *FGF5* rs1458038, *CYP17A1* rs1004467, *CSK* rs1378942, and *CSK-ULK3* rs6495122, showed significant association in either binary trait analyses (Tables S9) or quantitative trait analysis (Table 3 and S10). The most significant association was observed with *FGF5* rs1458038; this yielded a P value of 1.6×10^{-8} (+1.33 mm Hg) with SBP and 1.8×10^{-7} (+0.73 mm Hg) with DBP in the Millennium GPJ cohort, and the effect size was greater than that of Europeans (Table 3). Meta-analysis of both study panels with data from Global BPgen indicated further significant associations.

Multiple Regression Analysis for BP Trait and Hypertension in Japanese

To clarify whether the 4 susceptibility SNPs (*ATP2B1*, *FGF5*, *CYP17A1*, and *CSK*) were independently associated with BP traits and hypertension, multiple regression analysis was performed with possible covariates (Table S11). After adjustment for age, age^2, sex, BMI, and drinking habits, this analysis confirmed that all 4 of the SNPs were independent determinants for both BP traits and hypertension.

Combined Effect of Risk Genotypes on Hypertension

A risk score for 4 susceptible genotypes was calculated to evaluate their combined effects on hypertension. ORs asso-

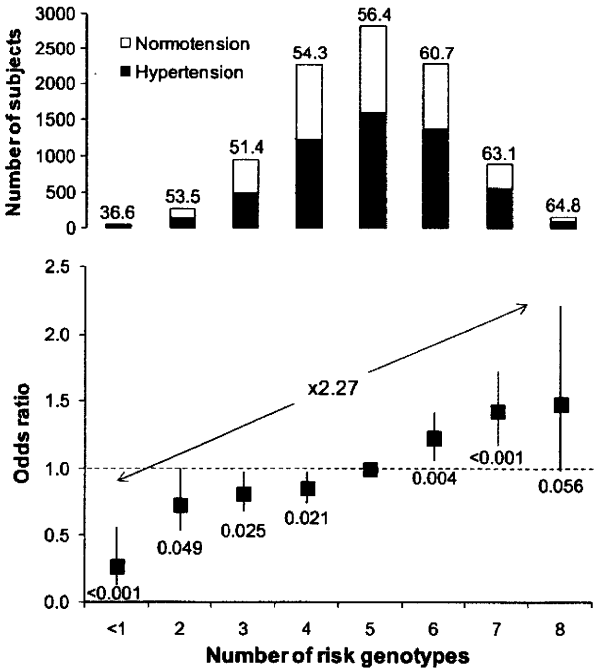

Figure 2. ORs for hypertension according to the number of risk genotypes Number of risk genotype was calculated by the following 4 SNPs: *ATP2B1* rs1105378, *FGF5* rs1458038, *CYP17A1*, rs1004467, and *CSK* rs1378942. Hypertensive subjects were defined as being treated with antihypertensive medication, SBP ≥140 mm Hg, or DBP ≥90 mm Hg; normotensive subjects were defined as all not treated with antihypertensive medication, SBP ≤120 mm Hg, and DBP ≤85 mm Hg.[2] Adjusted OR for hypertension and BP levels were calculated using logistic and linear multiple regression analysis, adjusting for sex, age, age^2, BMI, and cohort variables. Frequency of hypertension and *P* values for the hypertension odds are shown in the top of column and the bottom of square, respectively.

ciated with increasing number of risk genotypes in a covariates adjusted logistic regression model are depicted in Figure 2 (overall P value was 5.4×10^{-5}). Compared with the reference group (5 risk genotypes), individuals carrying 7 or 8 risk genotypes had higher risk (OR: 1.43 [95% CI: 1.20 to 1.72]; $P=1.0\times10^{-4}$) in contrast to the lower OR of individuals with ≤2 risk genotypes (OR: 0.63 [95% CI: 0.47 to 0.85]; $P=0.020$). The OR of the high-risk group was raised to 2.27 (95% CI: 1.65 to 3.12; $P=4.6\times10^{-7}$) compared with the lowest risk group. Adjusted per-allele OR for hypertension was 1.17 (95% CI: 1.12 to 1.21; $P=4.0\times10^{-15}$). The distribution of the Japanese population sample among the number of risk genotypes is shown in Figure S4.

Discussion

The present study has identified SNPs located upstream or within the *ATP2B1* gene as strong susceptibility polymorphisms for hypertension in Japanese. These are findings that have also been reported recently in individuals of European descent[3] and in Koreans.[4] Although numerous studies have attempted to identify genetic markers for hypertension over the past 2 decades, there has been little cross-validation of loci in different ethnic groups so far except for mendelian forms of hypertension. The SNPs in *ATP2B1* identified in this study showed significant association in large-scale studies in populations with different ancestries and using different discovery approaches, including GWAS in the CHARGE consortium and the Korean study and an independent candidate gene analysis in our present study. Similar findings in different ethnic groups with different methods further strengthen these findings and indicate the *ATP2B1* gene region as a susceptibility locus of likely global significance for BP variation and development of hypertension. Two replication results very recently reported by another Japanese group[12] and a Korean group[13] also indicated the disease susceptibility of *ATP2B1* SNPs located in the same LD block.

No biological data have been provided whether SNP rs1105378 or other SNPs in strong LD have any effect on the transcriptional activity or transcriptional regulation of the *ATP2B1* gene. Furthermore, although alternative splicing has been found to generate several variants of *ATP2B1* mRNA,[14] the SNP associations that we have observed do not shed light on whether this is a potential mechanism for affecting BP. Our data first showed that the effect of SNPs on ATP2B1 gene expression levels is a potential mechanism by which disease-associated SNP alleles cause the phenotypic changes. Changes in the *ATP2B1* gene product levels are involved in BP regulation. We found no microRNA harboring rs11105378 in the miRBase database.[15]

The *ATP2B1* (so-called *PMAC1*) gene encodes the plasma membrane calcium ATPase isoform 1, which removes bivalent calcium ions from eukaryotic cells against very large concentration gradients and plays a critical role in intracellular calcium homeostasis. Although pathophysiological implications of *ATP2B1* gene products on the development of hypertension are uncertain, it has been reported that inhibition of *ATP2B1* by the selective inhibitor caloxin 2A1 showed endothelium-dependent relaxation of rat aorta by increasing cytosolic Ca^{2+} concentration and consequent activation of endothelial NO synthase.[16] Other information on the role of *ATP2B1* has been obtained from experiments using bladder smooth muscle cells: contractility measurements on these cells have documented the important role of *ATP2B1* in the extrusion of Ca^{2+} after carbachol stimulation or depolarization with potassium chloride.[17] These reports suggest altered vascular reactivity as a plausible explanation for disease susceptibility of *ATP2B1* gene.

In mammals, calcium ATPase isoforms are encoded by ≥4 separate genes (*ATP2B1* to *ATP2B4*).[18] It has been reported that overexpression of the human *ATP2B4* gene in arterial smooth muscle cells in mice increases vascular reactivity and BP partly because of negative regulation of neuronal NO synthase.[19] We, therefore, examined the possible association of *ATP2B4* gene polymorphisms with hypertension by using the screening panel. However, no significant correlation was observed in the 17 SNPs analyzed, which were selected by reference to the HapMap database. The pathophysiological association of plasma membrane Ca^{2+} pump with BP regulation may be isoform specific.

Numerous studies, including the recent GWAS,[3–6] have attempted to identify genetic variations associated with human BP levels. At present, it is not clear to what extent findings from GWAS in one population can be extrapolated

to other populations with different lifestyles and genetic background. However, the present study provides a cross-validation of 4 of 7 SNPs (most likely representing 3 of 6 independent signals) derived from European GWAS. Replication studies in other Japanese[12] and Korean[13] populations also reported the cross-validation of European GWAS-derived SNP. Conservation of susceptible loci for hypertension was independent of ethnic background. This finding suggests an existence of unidentified common etiology of essential hypertension in relation to the susceptible genes and their physiological pathways.

Although individual common genetic variants confer a modest risk of hypertension, their combination showed a large impact on hypertension. The genetic risk score was associated with ≤2.27-times greater odds for hypertension. Similar observations have been found in other common diseases and multifactorial phenotypes, including, for example, type 2 diabetes mellitus,[20] serum lipid levels,[21] and serum uric acid levels.[22] We reported previously that the findings of the cross-sectional analysis revealed a similar association in the longitudinal analysis[23]; the fat mass and obesity-associated gene polymorphism was an independent risk factor for the future development of obesity after adjustment for possible confounding factors. The present cross-sectional study cannot address the question of whether the *ATP2B1* polymorphism and other susceptible variants predict future development of hypertension. However, recent articles investigating a prognostic significance of susceptible variants for type 2 diabetes mellitus[24] and cardiovascular disease[25] showed poor predictive performance of common variants in spite of the high OR observed in subjects carrying multiple risk alleles. A small proportion of the genetically high-risk persons attributed to independent inheritance of risk alleles may make it difficult to discriminate intermediate-risk persons. Genetic information may be most useful to identify a high-risk individual's need for early intervention.

Several definitions of hypertension were used in this study to explore susceptible SNPs with modest effects and to further validate the susceptibility. Since it was expected to be underpowered to detect the effects of common variants in a dichotomized analysis with slightly elevated BP, subjects with high normal BP were excluded from the 65 347 case-control analyses. All of the alleles associated with hypertension in a dichotomized analysis (Table S7) were also associated with BP levels (Table 2). Our methodology may, thus, be appropriate to identify susceptible variants for hypertension.

Perspectives

We have identified SNPs located in the *ATP2B1* gene region as susceptibility loci for hypertension in Japanese using a multistage association study, an association that has now been confirmed across different ethnic groups. Differences in the ex vivo *ATP2B1* mRNA expression levels further supported the disease susceptibility of SNP rs1110578. We also replicated the susceptibility of the European GWAS-derived SNPs in Japanese. Because hypertension is a trait that is preventable by dietary and exercise interventions, early detection of at-risk populations using genetic information may be useful in preventing future hypertension-related diseases.

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Disclosures

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ORIGINAL ARTICLE
Hypertension and Circulatory Control

A Genome-Wide Association Study of Hypertension-Related Phenotypes in a Japanese Population

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Background: Large-scale genome-wide association studies (GWAS) have been successful in identifying genes that contribute to common diseases and phenotypes. A GWAS of hypertension-related phenotypes in a Japanese population was conducted in the current study.

Methods and Results: A total of 936 participants were recruited from the Suita Study and a GWAS with 538,732 single nucleotide polymorphisms (SNP) was performed. The phenotypes included were systolic and diastolic blood pressure (SBP and DBP), body mass index (BMI), waist-to-hip ratio (WHR), plasma renin activity (PRA), plasma aldosterone concentration (PAC), plasma brain natriuretic peptide (BNP) concentration and alcohol consumption (AC). The SNP exceeding the genome-wide significance level were subjected to subsequent association studies using samples available from the Suita Study and Nomura Study. There is no master gene in the Japanese population that profoundly affects SBP, DBP, BMI, WHR, PRA and PAC. AC was influenced by the functional polymorphism in *ALDH2*, which affected BP levels in men. The BNP concentration was influenced by a polymorphism in the 3' region of the gene encoding for BNP. However, this polymorphism did not influence blood pressure (BP). Six SNP were identified to be associated with hypertension in both the Suita and Nomura studies.

Conclusions: Although several candidate SNP relevant to hypertension and those influencing AC and BNP were identified, our middle-sized GWAS indicated that there is no master gene in Japanese people that profoundly affects BP-related phenotypes. (*Circ J* 2010; **74:** 2353–2359)

Key Words: Aldehyde dehydrogenase 2; Brain natriuretic peptide; Genetics; Hypertension; Single nucleotide polymorphism

Large-scale genome-wide association studies (GWAS) have proven to be successful in identifying genes that contribute to common diseases and phenotypes.[1–8] The GWAS approach has also been used to identify common genetic variations that influence blood pressure (BP) and/or hypertension status.[3,4,9–11] An early GWAS involving 2,000 participants with essential hypertension and 3,000 controls showed that none of the associations achieved genome-wide significance ($P<5.0\times10^{-7}$).[9] Although the 6 top-ranked single nucleotide polymorphisms (SNP) in that study ($P<10^{-5}$) were subsequently examined in 11,433 participants from the US National Heart, Lung, and Blood Institute-funded Family Blood Pressure Program, the previously observed associations were not replicated using the dichotomous trait.[12] A recently published report on GWAS for BP in 34,433 participants of European ancestry revealed genome-wide significant associations of 8 loci with systolic BP (SBP), diastolic BP (DBP), and/or hypertension status.[4] In a replication study conducted in non-European populations,[4] only 2 of the 12 SNP were confirmed in Indian Asians (13,889 participants), suggesting that the results obtained in European-based populations might not be directly applicable to populations of different ethnic backgrounds.

The purpose of the current study was to conduct a genome-wide screen for hypertension-related phenotypes in a Japanese population. We recruited 936 individuals who participated in the Suita Study and performed a genome-wide screening with 538,732 SNP. The phenotypes included in the present study were SBP, DBP, pulse pressure (PP), pulse rate (PR), body mass index (BMI), waist-to-hip ratio (WHR), plasma renin activity (PRA), plasma aldosterone concentration (PAC), plasma brain natriuretic peptide (BNP) concentration, smoking habit (SM) and alcohol consumption (AC). Some of the top-ranked SNP were subsequently sub-

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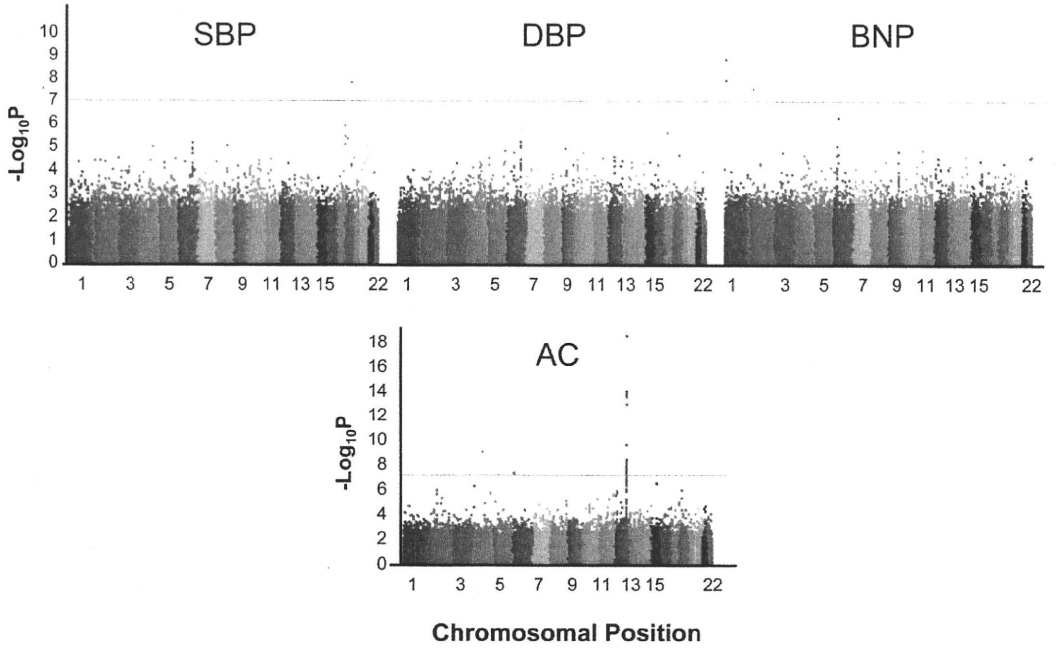

Figure 1. Manhattan plots for systolic blood pressure (SBP), diastolic blood pressure (DBP), B-type natriuretic peptide (BNP) and alcohol consumption (AC). The levels of significance ($-\log_{10}P$) derived from the association analysis of 368,274 single nucleotide polymorphism with each phenotype adjusted for covariates are plotted against chromosomal position. The genome-wide significance level is indicated by the dotted line.

jected to secondary screening using samples available from the Suita Study (n=3,228) and Nomura Study (n=2,895). We confirmed that AC and BNP are affected by a functional polymorphism in *ALDH2* and polymorphisms in the 3' region of *NPPB*, respectively.

Methods

Study Subjects

Suita Study Subjects (n=936, age range: 42–73 years, Table S1) who were not receiving medication for hypertension were selected from the Suita Study as the initial screening sample. The design of the Suita Study has been described previously.[13–15] In brief, the sample consisted of 14,200 men and women (30–79 years of age at enrollment, stratified by gender and 10-year age groups (10 groups with 1,420 participants in each group) who had been randomly selected from the municipal population registry. They were all invited by letter to attend regular follow-up examinations (every 2 years). Samples for genetic analysis were available for 3,228 participants (Table S1). BP was measured after 10 min of rest in the sitting position. SBP and DBP values were the means of 2 physician-obtained measurements. Physicians collected detailed personal medical information directly from the participants. The diagnosis of hypertension was based on BP measurement (SBP ≥140 mmHg or DBP ≥90 mmHg) or the current use of antihypertensive medications. Only those who gave written informed consent were included in the study.

Concentrations of PRA (n=2,328), PAC (n=2,382) and BNP (n=1,984) were measured in consecutive participants who were recruited during a defined period. PRA, PAC and BNP values were all log-transformed for statistical analyses. Residuals of SBP, DBP and PP were calculated by adjusting for age and BMI. Residuals of PR, BMI and WHR were calculated by adjusting for age and sex. Residuals of PRA, PAC and BNP were calculated by adjusting for sex and age. Residuals of PAC were also calculated by adjusting for PRA, sex and age (PAC corrected by PRA). Residuals of SM (number of cigarettes smoked per day) were calculated by adjusting for sex and age. Residuals of AC (grams of ethanol consumed per day) were calculated by adjusting for sex, height and age. The study protocol was approved by the Institutional Ethics Committee and the Committee on Genetic Analysis and Gene Therapy of the National Cerebral and Cardiovascular Center.

Nomura Study Study participants were selected from the residents of a community of 11,000 inhabitants in the Ehime Prefecture, a largely rural area located in western Japan.[16] Subjects were recruited during a community-based annual medical check-up in 2002 for self-employed people and included farmers, foresters, employees of small companies and the elderly without fixed employment. Baseline clinical characteristics (n=2,895, Table S2) were obtained from personal health records evaluated during the medical check-up. The values of SBP and DBP were measured after at least 5 min of rest. Hypertension status was defined as SBP ≥140 mmHg, DBP ≥90 mmHg or treatment with antihypertensive medication. Other characteristics, including smoking status and details of AC, medication, and history of cardiovascular disease, were investigated during individual interviews using a structured questionnaire. All study procedures were approved by the Ethics Committee of Ehime University Graduate School of Medicine, and written informed consent was obtained from all participants.

Table 1. Initial Screening Results and Validation of the Top-Ranked SNPs for SBP and DBP

Phenotype	SNP	Initial screening		Validation				
				P value*		P value†		
		Rank	$-\log_{10}P$	SBP	DBP	AA/AB/BB	AA/AB+BB	AA+AB/BB
SBP	rs1652080	1	7.87	0.0024	0.0035	0.0438	0.0129	0.7526
SBP	rs1468033	2	5.94	0.0012	0.0319	0.1911	0.5591	0.0689
SBP	rs9973037	5	5.32	0.000006	0.0018	0.0103	0.5013	0.0026
SBP	rs3778297	6	5.17	0.0085	0.0005	0.0269	0.1532	0.0093
SBP	rs6420481	7	5.14	0.0041	0.0460	0.7818	0.6422	0.5209
SBP	rs6013382	8	5.06	0.0031	0.0338	0.0190	0.0121	0.0455
SBP	rs1124697	10	5.04	0.1131	0.6117	0.0371	0.0653	0.0297
SBP	rs7692053	11	4.97	0.0021	0.0101	0.0553	0.0164	0.0472
SBP	rs7747460	12	4.95	0.0078	0.0002	0.0098	0.0975	0.0034
SBP	rs2076193	13	4.87	0.0070	0.0044	0.8202	0.7656	0.5348
SBP	rs3765258	15	4.69	0.0060	0.0005	0.0768	0.0258	0.3024
DBP	rs6505400	1	5.63	0.0472	0.0022	0.1359	0.2153	0.0646
DBP	rs10757609	5	4.92	0.0024	0.0202	0.0260	0.5359	0.0175

Due to the overlap between the top-ranked SNP for SBP and DBP, 13 SNP in total were selected for validation in the Suita Study.
*P values were obtained from association analyses with the residuals of SBP or DBP values among subjects who were not receiving antihypertensive medication; †P values were derived from association analyses using a dichotomous trait (hypertensive vs non-hypertensive) while adjusting for age and BMI; SNP shown in bold were further investigated in the Nomura Study (see Table 2 for more details).
SNP, single nucleotide polymorphisms; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Genotyping Assays

Genome-wide scans were performed for 936 Japanese participants using the Illumina Sentrix Human Hap550 BeadChip (538,732 SNP, Illumina Inc, San Diego, CA, USA), as previously reported.[17] Genotyping was performed by Illumina Inc. SNP with a call rate of less than 95% and/or with a minor allele frequency of less than 0.1 were excluded from the study, which left 368,274 autosomal SNP for analysis. Deviation from Hardy–Weinberg Equilibrium and the degree of linkage disequilibrium (LD) were analyzed using HaploView 4.0 (http://www.broad.mit.edu/mpg/haploview/). The top-ranked SNP were selected and genotyped in the available Suita samples (n=3,228) and then in the Nomura samples (n=2,895) for the validation of associations. Genotyping was performed by using TaqMan allelic discrimination assays (Applied Biosystems, Foster City, CA, USA), according to the manufacturer's instructions.

Statistical Analysis

Data are expressed as the mean±standard deviation. Continuous variables were tested for the normality of distribution, and those with skewed distributions were log-transformed. Residuals of various phenotypes, defined as the observed values minus the predicted values on the basis of confounding factors, were used for a genotype–phenotype association analysis by a one-way analysis of variance. The level of genome-wide significance, expressed as a $-\log_{10}P$ value, was adjusted for multiple testing by use of the Bonferroni correction and was set at a $-\log_{10}P$ value of >7.03. The odds ratio (OR) and 95% confidence interval (CI) were estimated by logistic regression analysis with adjustment for confounding factors. Statistical analysis was performed using the JMP statistical package 7.0 (SAS Institute, Cary, NC, USA).

Results

Figure 1 shows Manhattan plots of the genome-wide association analyses for SBP, DBP, BNP and AC. The top 1,000 SNP for SBP, DBP, PP, PR, BMI, WHR, PRA, PAC, PAC adjusted for PRA, BNP, SM and AC are listed in a supplementary file (Data S1). All data will be obtained at Genome Medicine Database of Japan (GeMDBJ, https://gemdbj.nibio.go.jp/dgdb/).

SBP, DBP, PP and PR

None of the SNP except for rs1652080 ($-\log_{10}P=7.87$) exceeded the genome-wide significance level for SBP. Of the top 15 SNP in SBP, 11 SNP ($4.69<-\log_{10}P<7.87$) listed in Table 1 were subjected to a validation study using the available samples from the Suita Study (total Suita samples). Four SNP (rs7224748, rs7225525, rs743093, rs9389418) were excluded due to tight LD with one of these 11 SNP. The initial associations became less significant in these 11 SNP. Due to the high prevalence of antihypertensive therapy (23.5% among women and 29.0% among men: Table S1), analyses were also carried out using the BP trait as a dichotomous variable (hypertensive vs non-hypertensive) (Table 1, see Table S3 for more details). We think the latter analysis is more reliable for identification of SNP influencing BP levels in populations with high prevalence of antihypertensive medications.

The top 5 SNP in DBP ($4.92<-\log_{10}P<5.63$, 3 SNP were included in the top 15 in SBP) were further genotyped for validation. Again, the initial associations became less significant in these 5 SNP (Table 1, see Table S3 for more details).

Other Phenotypes

None of the SNP exceeded the genome-wide significance level for BMI, WHR, PRA, PAC or PAC corrected by PRA. Two SNP (rs6676300 and rs198388) exceeded the genome-wide significance level for BNP; $-\log_{10}P$ values for rs6676300 and rs198388 were 8.89 and 7.97, respectively. These associations were validated in subsequent association analyses using the total Suita samples (Table S4). An increase in the sample size strengthened the associations for rs6676300

Table 2. Analysis of Candidate SNPs Related to Hypertension in the Suita and Nomura Studies

SNP	Sample	Hypertensive					Non-hypertensive					P value*			OR	95%CI
		Frequency			Allele frequency		Frequency			Allele frequency		AA/AB/BB	AA/AB+BB	AA+AB/BB		
		AA	AB	BB	A	B	AA	AB	BB	A	B					
rs1652080	Suita	25	247	961	0.12	0.88	19	405	1,547	0.112	0.888	0.0438	0.0129	0.7526		
Ch8_CCBE1	Nomura	13	300	1,219	0.106	0.894	11	211	1,115	0.087	0.913	0.0438	0.7479	0.0126		
	Combined											0.0294	0.0259	0.0597	1.831	1.073–3.140
rs3765258	Suita	701	456	78	0.752	0.248	1,019	809	143	0.722	0.278	0.0768	0.0258	0.3024		
Ch6_MAP7	Nomura	842	602	98	0.741	0.259	759	480	102	0.745	0.255	0.1205	0.6327	0.0780		
	Combined											0.1147	0.2041	0.0597	1.144	0.995–1.315
rs3778297	Suita	93	492	645	0.276	0.724	177	873	918	0.312	0.688	0.0269	0.1532	0.0093		
Ch6_MAP7	Nomura	109	618	809	0.272	0.728	114	472	757	0.261	0.739	0.0228	0.0985	0.1184		
	Combined											0.0950	0.0305	0.4148	0.793	0.643–0.979
rs6013382	Suita	552	544	131	0.672	0.328	976	812	173	0.705	0.295	0.0190	0.0121	0.0455		
Ch20_ZFP64	Nomura	692	669	175	0.668	0.332	621	593	127	0.684	0.316	0.2327	0.5989	0.0877		
	Combined											0.0109	0.0276	0.0084	0.776	0.643–0.937
rs7692053	Suita	213	583	434	0.41	0.59	293	987	689	0.399	0.601	0.0553	0.0164	0.0472		
Ch4_PCDH18	Nomura	297	739	494	0.436	0.564	230	628	482	0.406	0.594	0.0291	0.0431	0.0207		
	Combined											0.0042	0.0017	0.0413	1.273	1.095–1.481
rs7747460	Suita	97	495	640	0.28	0.72	190	882	901	0.32	0.68	0.0098	0.0975	0.0034		
Ch6_MAP7	Nomura	111	630	794	0.278	0.722	119	478	748	0.266	0.734	0.0181	0.0636	0.1496		
	Combined											0.0439	0.0136	0.2553	0.773	0.629–0.949
rs9973037	Suita	113	558	562	0.318	0.682	169	804	989	0.291	0.709	0.0103	0.5013	0.0026		
Ch18_CDH2	Nomura	122	631	781	0.285	0.715	111	530	702	0.28	0.72	0.8566	0.5787	0.8933		
	Combined											0.0847	0.8892	0.0309	1.133	1.011–1.268
rs1075609	Suita	45	341	846	0.175	0.825	58	621	1,289	0.187	0.813	0.0260	0.5359	0.0175		
Ch16_WWOX	Nomura	91	518	926	0.228	0.772	74	469	794	0.231	0.769	0.8071	0.9244	0.5566		
	Combined											0.0561	0.6414	0.0335	1.136	1.010–1.279
rs1460138†	Suita	177	575	474	0.379	0.621	227	921	814	0.35	0.65	0.0033	0.0013	0.0468		
Ch4_PCDH18	Nomura	253	705	568	0.397	0.603	185	598	546	0.364	0.636	0.0388	0.0353	0.0387		
	Combined											0.0002	0.0001	0.0040	1.374	1.166–1.620

*P values were derived from the association analyses using a dichotomous trait (hypertensive vs non-hypertensive) with adjustment for age and BMI, †rs1460138 was added after additional genotyping of 5 SNP around rs7692053.
OR, odds ratio; CI, confidence interval. Other abbreviations see in Table 1.

and rs198388; $-\log_{10}P$, 13.24 for rs6676300 and 12.71 for rs198388. The minor alleles of rs6676300 and rs198388 were associated with higher levels of BNP. Two SNP (rs2789567 and rs11717167) exceeded the genome-wide significance level for a SM. However, the initial association was not reproduced in the subsequent validation study (data not shown). As clearly shown in the Manhattan plot for AC (Figure 1), 20 SNP on Ch12 exceeded the genome-wide significance level, with $-\log_{10}P$ values ranging from 7.23 to 18.16. As our research group has previously reported using the Suita Study,[18] rs671 located in the *ALDH2* gene at Ch12q24.2 was identified as a casual polymorphism. This Glu487Lys missense mutation confers a low Km for ALDH2 enzymatic activity. Although rs671 is the major determinant of AC, especially among Japanese men, rs671 was not covered on the Illumina chip used in the present study. We used rs671 for the rest of our analyses. The influence of this polymorphism on drinking behavior was reconfirmed in the Nomura Study (Table S5). Sex-specific analyses revealed that the association was stronger in men than in women in both of the studies. Among the top-ranked SNP for AC, 2 SNP were not located on chromosome 12; rs4859731 on chromosome 4 ($-\log_{10}P$, 8.88) and rs482079 on chromosome 6 ($-\log_{10}P$, 7.19). The initial associations observed for these 2 SNP were not reproduced in the subsequent validation study (data not shown).

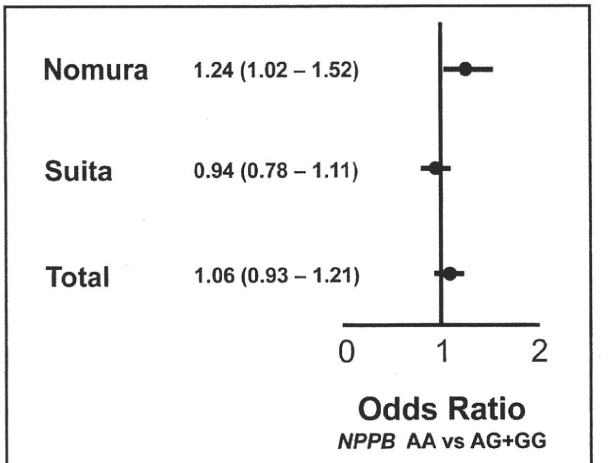

Figure 2. *NPPB* rs632793 genotypes and hypertension. Major homozygotes (AA) were compared to those with at least 1 minor allele (AG+GG) by multiple logistic regression analyses. Odds ratios and 95% confidence intervals are shown. Age, body mass index, and study population (Nomura or Suita) were included as confounding factors. No significant effect of this polymorphism on hypertension as a categorical variable (hypertensive vs non-hypertensive) was observed even when the 2 study populations were combined.

Further Analyses of rs6676300 and rs671

As summarized in Table S4, we genotyped several SNP around rs6676300 using the total Suita samples. The SNP rs632793 was found to be the most significant SNP for BNP, with a $-\log_{10}P$ value of 14.67. Age, BMI, PP, hematocrit, PRA and rs632793 influenced plasma BNP levels ($P<0.0001$, respectively). Several polymorphisms in this region have been reported to be associated not only with plasma BNP and atrial natriuretic peptide levels but also with BP levels.[19] However, the rs632793 polymorphism was not associated with the prevalence of hypertension (Figure 2) or BP levels (Table S3) in the Suita Study. The sample size of the Suita Study might have been too small to detect the influence of the rs632793 polymorphism on BP. Thus, the influence of this polymorphism on BP was also assessed in the Nomura Study to increase the statistical power. However, no significant influence of this polymorphism on BP was observed even after the 2 study populations were combined (Figure 2 and Table S3).

Excessive alcohol intake conferred by the *ALDH2* genotype has been reported to be associated with hypertension in

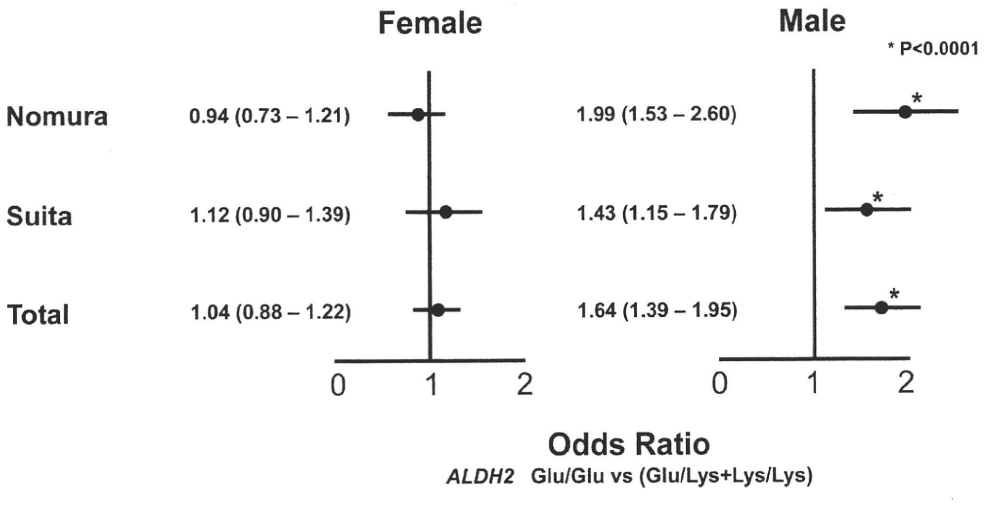

Figure 3. *ALDH2* rs671 genotypes and hypertension. Major homozygotes (Glu/Glu) were compared to those with at least 1 low-risk allele (Glu/Lys and Lys/Lys) by multiple logistic regression analyses. Sex-specific analysis was performed. Age, body mass index and study population (Nomura or Suita) were included as confounding factors. Odds ratios and 95% confidence intervals are presented.

men.[18,20] This was confirmed among men in the Nomura Study (Figure 3). When participants with the Glu/Glu genotype were compared to those with at least 1 low-risk allele (Lys), the OR for the prevalence of hypertension was 1.64 (95%CI, 1.39–1.95; $-\log_{10}P=8.03$; Figure 3) in men. The inclusion of AC in the multiple logistic regression analysis attenuated the significance of the ALDH2 genotype ($-\log_{10}P=4.36$).

Further Analyses of Top-Ranked SNP in SBP and DBP

As described above, the initial associations of the top 13 SNP in SBP and DBP became less significant with an increase in the sample size. However, there were nominally significant associations between 8 SNP and BP traits in the total Suita samples, when residuals of SBP ($P<0.01$) and a dichotomous trait (hypertensive vs non-hypertensive) ($P<0.05$) were both taken into account. Therefore, we assessed these 8 SNP in the Nomura Study (Table 2 and Table S3). The associations with hypertension as a categorical variable (hypertensive vs non-hypertensive) became more significant with an increase in the sample size in the 5 SNP (rs1652080, rs3778297, rs6013382, rs7692053 and rs7747460). Although they were weakened by an increase in the sample size, nominal P-values for the other 2 SNP (rs1075609 and rs9973037) were still below 0.05. However, the reproducibility of the associations with BP values was not high (Table S3). Only 2 SNP (rs1652080 and rs6013382) gave associations of $P<0.05$ with BP levels in the Nomura Study. The SNPs rs3765258, rs3778297 and rs7747460 were all located on chromosome 6q23 and there was tight LD among them. Additional typing of 5 SNP around rs7692053 indicated that hypertension as a dichotomous trait (hypertensive vs non-hypertensive) was most strongly associated with rs1460138 (OR=1.374, P=0.0001, Table 2).

Identification of associations with various intermediate phenotypes might be useful for elucidating the pathophysiological significance of SNP. Significant associations with renin profiles were observed for 4 SNP (rs10757609, rs3778297, rs6013382 and rs7747460, see Table S6). The SNP rs1460138 (in LD with rs7692053) significantly influenced the relationship between ethanol consumption and BP levels (Figure S1).

Discussion

Although the genetic architecture of many common diseases has been determined by GWAS, there has been limited success with GWAS for hypertension. Recently, 2 large GWAS of BP levels have been reported. The first included 34,433 participants of European ancestry for genome-wide screening and 71,225 participants from European-based populations and 12,889 non-Europeans for validation.[4] The other study included 29,136 particpants for genome-wide association analysis and 34,433 participants for replication.[3] Despite the large sample sizes used in these studies, only 2 chromosomal regions have been consistently reported to affect SBP (*CYP17A1* and *SH2B3*). Top-ranked SNP that affected SBP, DBP and/or hypertension status in 1 study were not reproduced in the other study.[3,4,9–11] While this might indicate that the contribution of a common polymorphism in a single gene is too small to be detected (lack of sample power), it could also be due to heterogeneity of the etiology of hypertension or the importance of rare functional mutations.

The present study was the first GWAS of hypertension-related phenotypes conducted in a Japanese population. Only one SNP (rs1652080) exceeded the genome-wide significance level for SBP. Moreover, the initially observed associations were not strengthened in the subsequent validation studies. These observations suggest that the sample power of the present GWAS might have been too low to identify SNP that influence SBP or DBP, and that there are no master genes that profoundly affect SBP or DBP in Japanese. This situation appears to be true for PP, PR, BMI, WHR, PRA, PAC, and PAC corrected by PRA and SM.

In contrast, the SNP that affect AC and BNP exceeded the genome-wide significance level, and subsequent validation studies confirmed that these associations were valid.

The SNP rs671, a missense mutation in *ALDH2*, has been repeatedly reported to influence AC in Japanese, particularly among men.[18,20] The present GWAS indicated that no other master genes influenced AC in this population. The Suita and Nomura Studies have confirmed that this functional polymorphism is associated with the prevalence of hypertension in men through its effect on alcohol intake. Although the inclusion of AC in the model for the multiple logistic regression analysis attenuated the significance of the *ALDH2* genotypes, it could still be considered that *ALDH2* genotypes might influence BP, independent of AC. We speculate that the underreporting of AC, which often occurs among heavy drinkers, might have exaggerated the influence of the *ALDH2* genotype on BP.

The SNP at the *NPPA-NPPB* locus have been reported to influence plasma BNP levels and BP.[19] The present study confirmed that plasma BNP levels were profoundly influenced by rs632793 in a Japanese population. However, no significant influence of the rs632793 polymorphism on BP was observed in our 2 study populations. A previous study reported that the SNP rs5068 and rs198358 affected SBP by only 0.9–1.5 mmHg.[19] The SNP rs5068 has been reported not to exist in Asians. The SNP rs198358 was in tight LD with rs6676300. No significant influence of rs6676300 on BP was observed in the Suita Study. It is likely that a much larger study population will be necessary to confirm the possible influence of rs632793 or other SNP at the *NPPA-NPPB* locus on BP in Japanese. It can also be speculated that a prolonged elevation of plasma BNP levels might reduce sensitivity to BNP and this polymorphism might not influence BP levels.

Validation studies of top-ranked SNP with regard to their influence on SBP and DBP showed that none of the SNP achieved genome-wide significance levels. However, 8 SNP or 6 chromosomal regions (including rs1460138 added after additional typing around rs7692053) exhibited nominal P-values for hypertension (as a categorical variable) below 0.05 after we combined the 2 study populations (Table 1). Notably, the associations became stronger when we combined the 2 study populations for 5 of the 8 SNP (or 3 out of the 6 chromosomal regions).

Identification of associations not only with BP but also with various intermediate phenotypes can be very important for determining how such genetic susceptibility contributes to hypertension and what strategies might be useful in terms of treatment and/or prevention. In this sense, the associations of the 4 SNP (rs10757609, rs3778297, rs6013382 and rs7747460, Table S6) with renin profiles are interesting. They might contribute to salt sensitivity in Japanese. Likewise, the significant influence of rs1460138 on the relationship between ethanol consumption and BP levels was intriguing (Figure S1). Participants with the CC+AC genotype of rs1460138 should be advised not to drink too much. The SNP identified in the present study are excellent candidates for hypertension-susceptible genes in a Japanese population

and merit further investigation using much larger study populations.

GWAS is regarded as a useful method to identify genes or genetic loci involved in pathogenic pathways without a priori biological knowledge. Concerning the genetic determinants of inter-individual differences in drug responses, pharmacogenetic studies have been rigorously performed.[21,22] This pharmacogenetic approach might be further expanded with the discovery of new genes or genetic variants through GWAS.

The present study was the first GWAS of hypertension-related phenotypes in a Japanese population. Even though our genome-wide screen was conducted in a medium-sized population, SNP that influence AC and BNP were confirmed in the present study. It appears that there is no master gene in Japanese that profoundly affects SBP, DBP, PP, PR, BMI, WHR, PRA, PAC, or PAC corrected by PRA or SM. However, several candidate SNP relevant to hypertension in Japanese were identified.

Study Limitations

To identify polymorphisms with minor to moderate influence, screening for genome-wide association analyses should be performed with a much larger sample size and subsequent validation studies should be done in more than tens of thousands of participants. However, because the influence of a genetic susceptibility will be modulated by interaction with various environmental factors, thorough and accurate information on individual clinical, behavioral, and habitual characteristics will be necessary. These antithetical difficulties might be resolved by the establishment of an international consortium on various phenotypes.

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Conflict of interests/Disclosures

None.

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Supplymentary files

Table S1. Clinical Characteristics of the Study Populations: the Suita Study

Table S2. Clinical Characteristics of the Study Population: the Nomura Study

Table S3. Validation of the Top-Ranked SNP for SBP and DBP and Candidate SNP Relevant to Hypertension

Table S4. Validation of BNP-Associated SNP, rs6676300 and rs198388, and Their Nearby SNP

Table S5. The Effect of rs671 Genotypes on Alcohol Consumption

Table S6. Renin-Aldosterone Profile and Candidate SNP Relevant to Hypertension

Figure S1. Multiple logistic regression analyses indicated that hypertension status (hypertensive vs non-hypertensive) was associated with age (P<0.0001), BMI (P<0.0001), study population (Nomura or Suita Study) (P<0.0001), ethanol consumption (P<0.0001), rs1460138 (AA vs AC+CC, P=0.0007), and the interaction between ethanol and rs1460138 (P=0.0019).

Data S1. The top 1,000 SNP for SBP, DBP, PP, PR, BMI, WHR, PRA, PAC, PAC adjusted for PRA, BNP, SM and AC are listed in a supplementary file.

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Guidelines for Diagnosis and Treatment of Patients With Vasospastic Angina (Coronary Spastic Angina) (JCS 2008)

– Digest Version –

JCS Joint Working Group

Table of Contents

(*Circ J* 2010; **74:** 1745–1762)

Preface: In Formulating the Guidelines

Coronary spasm is defined as a condition in which a relatively large coronary artery running on the surface of the heart transiently exhibits abnormal contraction. If a coronary artery is completely or nearly completely occluded by spasm, transmural ischemia occurs in the region perfused by the artery, which in turn causes anginal attacks with ST elevation on the ECG. If a coronary artery is partially occluded or diffusely narrowed by spasm, or if it is completely occluded by spasm but sufficient collateral flow has developed distally, non-transmural ischemia occurs, causing anginal attacks with ST depression on the ECG. These pathological conditions are collectively termed vasospastic angina (also termed coronary spastic angina), as a type of angina caused by coronary spasm. Variant angina, characterized by ST elevation during anginal attacks, is another type of vasospastic angina. Coronary spasm has been shown to play key roles in the onset of not only variant angina but also rest angina, effort angina, acute myocardial infarction, and other related conditions.[1] The mechanism of involvement of coronary spasm in the onset of acute coronary syndrome is now being elucidated.[2–4]

In drawing up the present guidelines, cases of vasospastic angina were categorized into three classes as described below. Please note that no evidence levels are established for the guidelines, since no large-scale clinical studies of this condition have been performed.

Classification of Recommendations

Class I: The benefits and efficacy of a method of evaluation or treatment have been demonstrated or are widely approved.

Class II: Some discrepancy exists in findings or opinions regarding the benefits and efficacy of a method of evaluation or treatment.

Class IIa: As judged from available findings and opinions, a method of evaluation or treatment is likely to be beneficial and effective.

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Address for correspondence: Scientific Committee of the Japanese Circulation Society

This English language document is a digest version of Guidelines for Diagnosis and Treatment of Patients with Vasospastic Angina (Coronary Spastic Angina) reported at the Japanese Circulation Society Joint Working Groups performed in 2006–2007 (*Circulation Journal* 2008; **72**(Suppl IV): 1239–1252).

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Class IIb: As judged from available opinions, neither the benefits nor the efficacy of a method of evaluation or treatment have been well established.

Class III: A method of evaluation or treatment has been demonstrated to be useless and possibly harmful at times, or its harmfulness has been widely agreed upon.

The primary goal of formulating the present guidelines is to establish a definition of vasospastic angina, and to provide diagnostic criteria for this condition. These guidelines are composed of standards generated on the basis of a great deal of evidence. Individual patients have their own specific clinical features, and you are encouraged to use the guidelines with this fact in mind.

The present guidelines provide guidance on the diagnosis and treatment of patients with vasospastic angina for physicians in clinical practice. The final decisions regarding diagnosis and treatment should be made by the attending physicians after the pathologic condition of each patient has been individually determined. In addition, even if a diagnosis or treatment not in conformity with the guidelines is implemented, it should be noted that determination of treatment by attending physicians based on the specific conditions and circumstances of their patients should take precedence over the guidelines, and that the present guidelines provide no grounds for argument in cases of legal prosecution.

We hope that these guidelines will be useful in the diagnosis and treatment of patients with vasospastic angina by cardiologists and all other physicians.

I Overview

1. Definition and Pathology

1 Characterization of Coronary Spasm in Ischemic Heart Disease

(1) Characterization of Coronary Spasm in Terms of the Etiology of Angina

In coronary spasm, sudden excessive coronary vasoconstriction produces a transient reduction of blood flow, resulting in myocardial ischemia (supply ischemia/primary angina). Although coronary spasm occurs mainly in large coronary arteries running on the surface of the heart, it is also known to occur in the coronary microvasculature of the myocardium. Coronary spasm is not always preceded by elevations of blood pressure and heart rate, which increase myocardial oxygen consumption. In this regard, coronary spasm is a pathological condition that is clearly distinguishable from demand ischemia/secondary angina represented by effort angina.

Coronary spasm develops in sclerotic lesions of varying severity. Even when no stenotic lesions are visible on coronary angiography, intravascular ultrasound (IVUS) reveals clear arteriosclerotic lesions in locations consistent with regions of coronary spasm.[5] Reduction of blood flow due to coronary spasm activates platelets and the coagulation system,[6] causing vascular smooth muscle cell proliferation.[7] It has in fact been revealed by evaluation using quantitative coronary angiography that the locations of coronary spasm induced in provocation tests were particularly susceptible to progression of arteriosclerosis.[8,9]

(2) Characterization of Coronary Spasm in Acute Coronary Syndrome

It was reported as early as the 1970s that coronary spasm can trigger not only angina but also myocardial infarction. There have been patients with acute myocardial infarction in whom emergent coronary angiography revealed extremely mild organic stenosis, as well as patients with complete coronary occlusion which exhibited recanalization after administration of nitrates alone. Recently, unstable angina, acute myocardial infarction, and sudden ischemic cardiac death have been referred to collectively as acute coronary syndrome. This is because these diseases share the pathological finding of rapid progression of coronary lesions, ie, disruption of coronary atheroma (plaque) and the resulting thrombus formation.[10] Coronary plaques are observed in the form of local thickening of the intima, and are structurally characterized by the accumulation of foamy macrophages forming a lipid core covered by a fibrous cap of connective tissue and smooth muscle cells. It has been hypothesized that if a tear occurs in this cap, the highly thrombogenic plaque content becomes exposed to the blood flow and rapidly forms thrombi that obstruct the vascular lumen. Plaques more likely to be ruptured are termed vulnerable plaques; they are often characterized by high lipid content and a thinned fibrous cap, and tend to be large.

It has been suggested that coronary spasm is a cause of rupture of vulnerable plaques. Investigations of coronary lesions in autopsies have demonstrated that spasm causes endothelial cell derangement and fibrous cap rupture, resulting in the protrusion of the plaque content exposed to the vascular lumen, where thrombi are produced.[11] In addition, coronary spasm is accompanied by hypercoagulation,[12] decreased fibrinolytic activity,[13] and activation of platelets and adhesion molecules,[14] resulting in a thrombophilic state in acute coronary syndrome. Although plaque stabilization (prevention of rupture) and antithrombotic therapy are important in the prevention and treatment of acute coronary syndrome, prevention of coronary spasm is also important, particularly in Japanese, in whom the prevalence of coronary spasm is higher than in western countries.

2 Diagnostic Criteria

At present, vasospastic angina is diagnosed in Japan using criteria independently adopted by individual institutions. In this background, the present guidelines are established to unify the diagnostic criteria with reference to previous reports and other findings. Yasue et al. state that vasospastic angina can be diagnosed even without performing coronary angiography, provided that anginal attacks disappear quickly upon administration of nitroglycerin, and that any one of the five conditions shown below is met: (1) attacks appear at rest, particularly between night and early morning; (2) marked diurnal variation is observed in exercise tolerance (in particular, reduction of exercise capacity in the early morning); (3) attacks are accompanied by ST elevation on the ECG; (4) attacks are induced by hyperventilation (hyperpnea); (5) attacks are suppressed by calcium channel block-

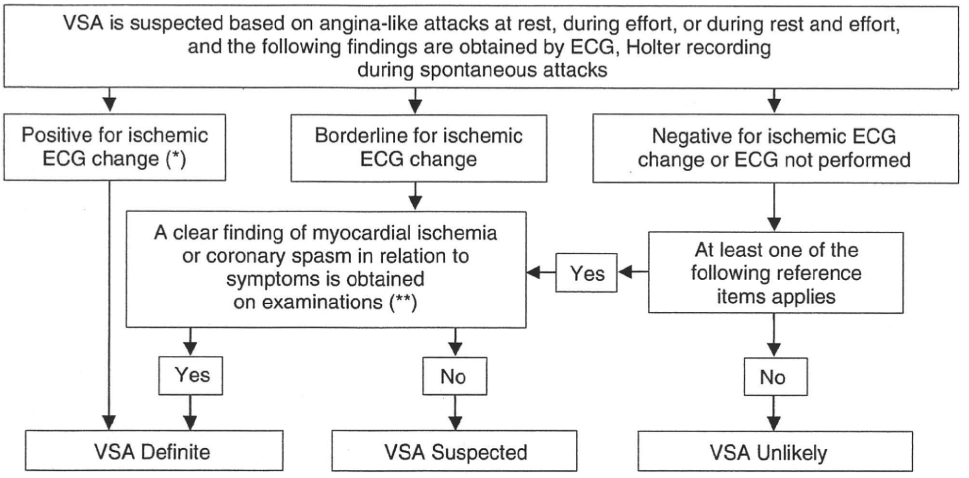

Figure 1. Flow chart of diagnosis of vasospastic angina (VSA). Reference items: An angina-like attack that disappears quickly upon administration of a nitrate, and that meets at least one of the following four items: 1) Appears at rest, particularly between night and early morning, 2) Marked diurnal variation in exercise tolerance is observed (in particular, reduction of exercise capacity in the early morning), 3) Induced by hyperventilation (hyperpnea), 4) Attacks are suppressed by calcium channel blockers but not by β-blockers. (*) Ischemic change is defined as a transient ST elevation of 0.1 mV or more, an ST depression of 0.1 mV or more, or new appearance of negative U waves, recorded in at least two contiguous leads on the 12-lead ECG. If the ischemic ECG change is prolonged, patients should be treated as directed in the guidelines for management of acute coronary syndrome. (**) Examinations include the drug-induced coronary spasm provocation test during cardiac catheterization and hyperventilation test. A positive finding for coronary spasm on coronary angiography in the acetylcholine- or ergonovine-induced coronary spasm provocation test is defined as "transient, total, or sub-total occlusion (>90% stenosis) of a coronary artery with signs/symptoms of myocardial ischemia (anginal pain and ischemic ECG change)".

ers but not by β-blockers.[15] In the present guidelines, reference items based on that opinion are included in the diagnostic criteria established for three grades: "Definite," "Suspected," or "Unlikely". The diagnostic criteria for vasospastic angina are provided below. A diagnostic flow chart is shown in Figure 1.

Diagnostic Criteria for "Definite/Suspected" Vasospastic Angina

If any one of the following conditions and one of the following requirements are met, Definite/Suspected vasospastic angina is considered present. If none of them is met, the condition is judged Unlikely to be vasospastic angina. Clinically, both Definite and Suspected vasospastic angina are diagnosed as vasospastic angina.

Conditions (any one of the three below)

1. Spontaneous attacks
2. Positive non-drug-induced coronary spasm provocation test (eg, hyperventilation test and exercise test)
3. Positive drug-induced coronary spasm provocation test (eg, acetylcholine provocation test and ergonovine provocation test)

Requirements

A. "Definite vasospastic angina"
The patient is considered to have Definite vasospastic angina when ischemic change is clearly observed on the ECG during attacks (*); when the ECG findings are borderline but a clear finding of myocardial ischemia or coronary spasm is obtained in examinations (**) and he/she has a history and symptoms during attacks that are consistent with vasospastic angina; or when, if there is no ECG change during attacks or if ECG examination has not been performed, at least one of the following reference items is met, and examinations (**) reveal a clear finding of myocardial ischemia or coronary spasm.

B. "Suspected vasospastic angina"
The patient is considered to have Suspected vasospastic angina when the ischemic change on ECG during attacks is in the borderline, and no clear finding of myocardial ischemia or coronary spasm is obtained in any examination (**); or when, if there is no change on the ECG during attacks or ECG examination has not been performed, one or more of the following reference items apply, and a clear finding of myocardial ischemia or coronary spasm cannot be demonstrated on any examination (**).

(*) Ischemic change is defined as a transient ST elevation of 0.1 mV or more, an ST depression of 0.1 mV or more, or new appearance of negative U waves, recorded in at least two contiguous leads on the 12-lead ECG. If the ischemic ECG change is prolonged, patients should be treated as directed in the guidelines for management of acute coronary syndrome.

(**) Examinations include the drug-induced coronary spasm provocation test during cardiac catheterization and hyperventilation test. A positive finding for coronary spasm on coronary angiography in the acetylcholine- or ergonovine-induced coronary spasm provocation test is defined as "transient, total, or sub-total occlusion (>90% stenosis) of a coronary artery with signs/symptoms of myocardial ischemia (anginal pain and ischemic ECG change)."[16–19]

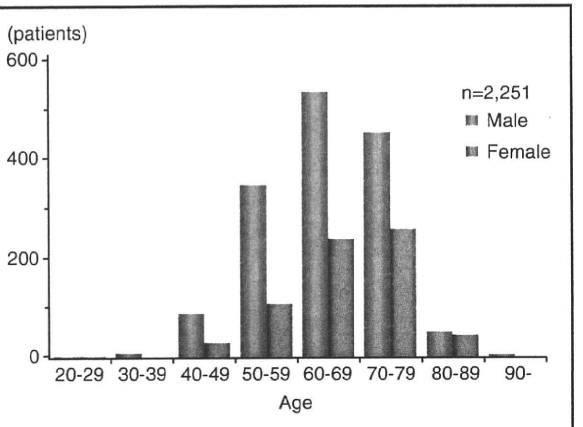

Figure 2. Age group distribution of angina in Japanese.

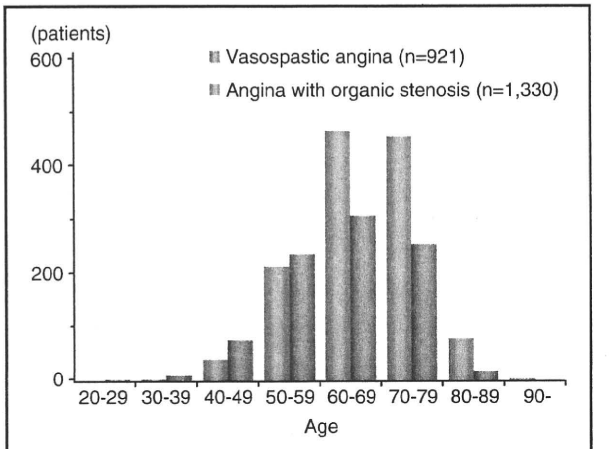

Figure 3. Number of patients with vasospastic angina and angina with organic stenosis according to age group.

Reference Items

An angina-like attack that disappears quickly upon administration of a nitrate, and that meets at least one of the following four items:

1) Appears at rest, particularly between night and early morning.
2) Marked diurnal variation in exercise tolerance is observed (in particular, reduction of exercise capacity in the early morning).
3) Induced by hyperventilation (hyperpnea).
4) Attacks are suppressed by calcium channel blockers but not by β-blockers.

2. Etiology and Epidemiology

1 Etiology

(1) Environmental Factors

(1) Smoking

A large number of coronary risk factors have been identified, including hypertension, lipid abnormalities, smoking, diabetes mellitus, and obesity. Of these, smoking is a well-recognized risk factor for coronary spasm.[20–23] In fact, many reports have shown that a high percentage of patients with vasospastic angina in Japan are tobacco smokers. Smoking is a controllable factor in preventing the development of coronary spasm; smoking cessation programs are thus indispensable in the treatment of coronary spasm.[24]

(2) Drinking

Patients with vasospastic angina in Japan include many habitual drinkers.[21] Alcohol promotes the urinary excretion of magnesium, which in turn is likely to lead to tissue magnesium deficiency. It has been shown that many patients with vasospastic angina have magnesium deficiency,[25] and it has been reported that intravenous administration of magnesium prevents hyperpnea-related attacks of coronary spasm.[26] Alcohol restriction is thus required in patients with vasospastic angina.

(3) Lipid Abnormalities

It has been reported that patients with vasospastic angina often have abnormalities of lipid metabolism and glucose metabolism as complications.[27–29] It has been suggested that oxidative stress may be associated with abnormalities of triglyceride metabolism, HDL cholesterol level reduction, and impaired glucose tolerance.

(4) Stress (Abnormal Autonomic Nervous System Function)

Attacks of coronary spasm is induced by a wide variety of stimuli that act on receptors on coronary smooth muscle cells, including those due to abnormal autonomic nervous system function.[30] In addition to its direct effect, ie, the release of vasopressor neurotransmitters such as noradrenaline, platelet activation via the sympathetic nervous system also causes the release of serotonin, a potent coronary constrictor. Many analyses focusing on heart rate changes have reported that in patients with vasospastic angina, as in those with other types of ischemic heart disease, parasympathetic nervous dysfunction tends to cause an imbalance in sympathetic and parasympathetic nerve, with predominance of sympathetic activity.[31,32]

(2) Genetic Factors

Since coronary diseases are often familial, and persons with no lifestyle-related risk factors can nevertheless develop them, it has been suggested that "genetic factors" may also be involved in their onset. In recent years, due to the remarkable advances in molecular biology, genes involved in the pathophysiology of various diseases have been cloned, and genom polymorphisms and variations have been identified; a great deal of research into multifactorial diseases has emerged, including that on lifestyle-related diseases, from molecular epidemiological perspectives. Single nucleotide polymorphism (SNP), in particular, is a form of polymorphism found in a large number of genes in the genome. It has been suggested that changes in the level of expression or function of protein molecules encoded by genes exhibiting SNPs may affect disease susceptibility. Analysis of the associations between SNPs and diseases is expected to elucidate the genetic factors involved in the pathophysiology of diseases, and enable their primary prevention by tailor-made medicine based on individual genetic features. In particular, coronary spasm occurs at higher incidence in Japanese than in Western individuals, and has been suggested to involving genetic factors. SNPs that have been identified as related to

Table. Characteristics of Coronary Spasm in Japan and Western Countries

	Japan	Western countries	P value
Total number of patients	752	586	
Female ratio (%)	13	22	<0.0001
Past history of myocardial infarction (%)	7	24	<0.0001
Organic coronary stenosis (%)	41	66	<0.0001
Multivessel disease (%)	24	44	<0.0001
Left ventricular dysfunction (%)	6	34	<0.0001
Multivessel spasm (%)	8	0	<0.0001
3-year prognosis myocardial infarction			
Incidence rate (%)	9	25	<0.0001
Mortality rate (%)	3	11	<0.0001

vasospastic angina include (1) the endothelial nitric oxide synthase (eNOS) gene Glu298Asp polymorphism,[33] (2) the eNOS gene-786T/C polymorphism,[34–38] (3) the eNOS gene intron 4b/a polymorphism,[39–41] and (4) the phospholipase C-δ1 (PLC-δ1) missense mutation (R257H).[42,43] Investigations using public databases have yielded reports on NADH/NADPH oxidase p22phox gene 242C → T polymorphisms (male), stromelysin-1 gene - 1171/5A → 6A polymorphisms (female), and interleukin-6 gene - 634C → G polymorphisms (female).[44]

2. Epidemiology: Prevalence and Race-Related Differences (Characteristics of Japanese)

(1) Prevalence of Vasospastic Angina

To determine the prevalence of vasospastic angina, a survey was conducted on 2,251 consecutive patients with angina (average age of 65.2 years) hospitalized in 15 major cardiovascular medical institutions in Japan in 1998.[45] Figure 2 shows the age group distribution of the disease in the study population. In Japan as well as Western countries, angina is more prevalent among males than females, and male prevalence increases with age. In females, the incidence of angina begins to rise at the average age of menopause, around 50 years, and sex-related differences in incidence no longer exist at above 80 years of age. In females, menopause represents a turning point in the onset of heart disease, and decreases in female sex hormones appear to play very important roles in this. Although the prevalence of vasospastic angina varies among institutions, about 40% of patients with angina studied had vasospastic angina. Analysis of the age group distribution of vasospastic angina revealed that prevalence tended to be higher in relatively young patients than in elderly ones (Figure 3).

(2) Race-Related Differences

Results of drug-induced coronary spasm provocation tests in Japan and Europe revealed higher incidences of coronary spasm in Japan than in Europe, although there were differences in both the route and dose level of administration of spasm inducers used.[19,46,47]

Characteristics of cases of vasospastic angina reported in Japan and Western countries are summarized in Table.[21,48–53] Although the female ratio is not high in either population, Japan has a lower female ratio than Western countries. Patients with a history of myocardial infarction, those with organic coronary stenosis, and those with multivessel diseases are prevalent among Western people. Reflecting these findings, left ventricular dysfunction is more prevalent in Westerners. In contrast to Westerners, Japanese patients with multivessel coronary spasm accounted for 8% of the study population. The mortality rate in patients with vasospastic angina is lower for Japanese; this is attributable to the fact that the incidence of myocardial infarction is higher in Westerners than in Japanese.

There are reports that coronary spasm was observed by provocation test early after the onset of myocardial infarction in 11 to 21% of Westerners[54,55] and 69% of Japanese.[56] The frequency of the total occlusion of the culprit lesions in the acute phase of myocardial infarction is significantly higher in Westerners (82%, 1,539/1,884) than in Japanese (64%, 296/465).[57] This finding suggests that coronary spasm may be involved in the development of myocardial infarction in many Japanese patients.

3. Pathophysiology

1 Involvement of Vascular Endothelial Cells

In patients with vasospastic angina, coronary spasm can be induced at high incidence without producing a change in systemic hemodynamics, by injecting acetylcholine directly into a coronary artery.[16] Acetylcholine dilates blood vessels when the vascular endothelium is normal, but if there is endothelial detachment or injury, it contracts blood vessels. This occurs because nitric oxide (NO), a potent smooth muscle relaxant, is secreted from endothelial cells, provided that the vascular endothelium is intact.[58–60] In the endothelium, NO is produced by eNOS, which releases NO upon activation by a wide variety of signals. On the other hand, eNOS becomes activated via calmodulin as a result of elevation of intracellular Ca^{2+} level by mechanical stimuli such as shear stress. The receptor-mediated vasodilation induced by vasoactive mediators such as acetylcholine, bradykinin, and serotonin activates receptors, G proteins, and phospholipase C (PLC) in vascular endothelium to produce inositol triphosphate (IP3) and release stored Ca^{2+} in cells. This receptor stimulation promotes Ca^{2+} inflow through ionic channels. Stimulation by physiological active substances such as acetylcholine, bradykinin, and insulin and mechanical stimuli such as shear stress also increase eNOS activity.[61]

Nitrates are metabolized to NO in the body, which in turn stimulates the soluble guanylate cyclase in vascular smooth muscle to increase the level of cyclic guanosine monophosphate (cGMP) and dilate blood vessels.[62] Because NO is produced and released from normal vascular endothelium, the hyperreactivity of spastic coronary arteries to nitroglycerin is probably due to a lack of baseline production or

release of NO from the endothelium in these arteries.[63]

2 Involvement of Vascular Smooth Muscles

Details of the mechanism of contraction of vascular smooth muscle were recently elucidated.[64,65] Specifically, in response to stimulation by constrictive vasoactive substances such as angiotensin II, G protein-coupled PLC in vascular smooth muscle cells is activated to produce IP3. IP3 opens Ca^{2+} channels on sarcoplasmic reticulum, a sarcoplasmic reticulum that stores Ca^{2+}, to release Ca^{2+} into the cytoplasm, resulting in increase in intracellular Ca^{2+} concentration. Ca^{2+} channels are also present in the cell membrane. They open in response to a wide variety of stimuli, followed by Ca^{2+} inflow from outside the cells. The Ca^{2+} release from the sarcoplasmic reticulum and Ca^{2+} inflow from outside the cells result in increased intracellular Ca^{2+} levels, and calcium ions bind to calmodulin to form Ca^{2+}/calmodulin complexes. These complexes bind to the catalytic subunit of myosin light chain kinase (MLCK) to convert MLCK from its inactive to its active form. Phosphorylation of the myosin light chain (MLC) by active MLCK activates Mg^{2+}-ATPase in the head of myosin, by actin, and vascular smooth muscle contracts. Subsequently, as the intracellular Ca^{2+} concentration falls, Ca^{2+} dissociates from calmodulin, and MLCK becomes inactivated. As a result, the activity of myosin light-chain phosphatase (MLCPh) becomes dominant, MLC undergoes dephosphorylation, and vascular smooth muscle relaxes.[66]

The phosphorylation of MLC is promoted and suppressed by MLCK and MLCPh, respectively. In addition, MLCPh has been shown to be suppressed by Rho-kinase. Rho-kinase is an important molecular switch that controls the contraction and relaxation of vascular smooth muscle independently of intracellular Ca^{2+} concentration. Upon stimulation by a vasopressor substance, Rho, a low-molecular-weight G protein, is activated via the G protein-coupled receptor, and Rho-kinase, one of its target proteins, is activated. The activated Rho-kinase phosphorylates the myosin-binding subunit (MBS) of MLCPh to inhibit its activity. As a result, the balance of MLCK/MLCPh activity is lost, phosphorylation of MLC is promoted, and vascular smooth muscle undergoes excessive contraction.[67,68]

II Diagnosis

1. Subjective Symptoms and Physical Findings

(1) Subjective Symptoms

(1) Characterized by vague pain that cannot be indicated by a single finger, with a sensation of compression, a pressing sensation, and a sensation of tightness in the precordium, especially in the center of the substernal region. Occasionally, symptoms develop in the upper abdomen.
(2) Appears at rest, with pain persisting for several to about 15 minutes. The pain often radiates to the neck, jaws, left shoulder, and elsewhere, occasionally accompanied by symptoms such as numbness and weakness of the left shoulder and upper arm.
(3) Anginal attacks due to coronary spasm often persist longer than effort anginal attacks due to organic stenotic lesions, and are sometimes accompanied by cold sweats and disturbance of consciousness including syncope.
(4) Can be induced by hyperpnea and drinking of alcohol.
(5) Fast-acting nitrates are remarkably effective against attacks of coronary spasm.
(6) Calcium channel blockers suppress attacks of coronary spasm.
(7) Attacks are often accompanied by arrhythmias; if they are complicated by complete atrioventricular block, ventricular tachycardia, or ventricular fibrillation, distur-

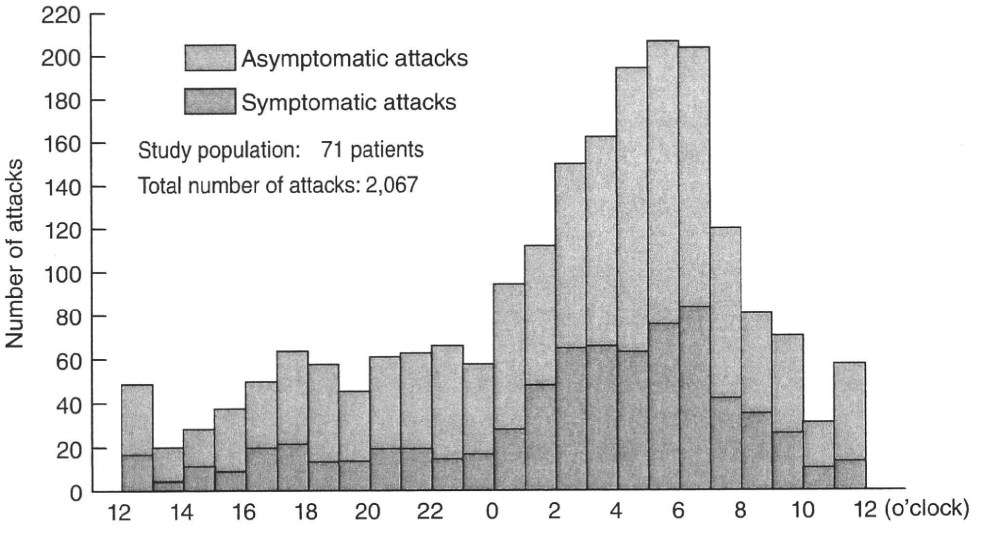

Figure 4. Diurnal variation of attacks of coronary spasm. Adapted from Yasue H, et al.[69]

bance of consciousness or syncope is observed.
(8) Attacks of coronary spasm typically occur at rest between night and early morning. They are usually not induced by daytime exercise. Diurnal variation with a peak between night and early morning is observed; 67% of attacks are asymptomatic episodes of myocardial ischemia without subjective symptoms (Figure 4).[69] Usually, attacks of vasospastic angina can be induced by even slight effort in the early morning, but are not induced by even strenuous effort in the afternoon or later in the day. Hence, diurnal variation is also observed in exercise tolerance in patients with vasospastic angina.
(9) Attacks of coronary spasm may occur frequently, ie, several times every day, or may not occur for several months to several years.

(2) Physical Findings
In auscultation during attacks, gallop rhythms and systolic murmurs are sometimes heard. These are caused by decreased wall motion, mitral regurgitation, and other changes resulting from ischemia. If symptoms disappear upon administration of a fast-acting nitrate or similar agent, these findings may also disappear. Hypotension may occur during attacks. In addition, since the arrhythmias developing in association with attacks include complete atrioventricular block, ventricular tachycardia, and ventricular fibrillation, they must be monitored for carefully.

2. Methods of Evaluation

1 Non-Invasive Evaluation

(1) ECG and Holter Recording
Class I
1. Two ECG records, obtained during an attack and after administration of fast-acting nitrate or just after symptom stabilization in cases in which vasospastic angina is strongly suspected based on subjective symptoms
2. Holter recording (multi-channel recording acceptable) for an extended period of time of 24 to 48 hours in cases in which vasospastic angina is strongly suspected based on subjective symptoms accompanied by syncope or palpitations without identifiable cause

Class IIa
Holter recording for 24 to 48 hours in cases in which it is difficult to record the ECG during attacks

Class IIb
1. ECG or Holter recording in patients in whom the likelihood of vasospastic angina is low, as judged from the patient's age, subjective symptoms, and background
2. 12-lead ECG records targeting time periods in which attacks are prevalent (in cases in which hyperventilation and exercise tests cannot be performed)

Class III
None

(1) Standard 12-Lead ECG
The ECG often exhibits normal findings in the absence of attacks. Hence, when symptoms occur frequently, a diagnosis can be established by recording the 12-lead ECG both in the presence and the absence of an attack. Typical ECG changes during attacks of vasospastic angina include ST elevation in leads corresponding to the culprit lesion and ST depression in contralateral leads. The diagnosis can be made because these findings normalize upon administration of a fast-acting nitrate. Many patients with vasospastic angina have moderate organic stenosis of the affected coronary arteries, and in some cases only ST depression is present in contiguous leads and the absence of ST elevation appears to depend on the severity of coronary spasm or ischemia. Other possible findings include the appearance of negative T waves in the culprit lesion during recovery from ischemia, and the new emergence of negative U waves during spasm.[48]

***Criteria for Positive Ischemic ECG Finding**
If an ST elevation of 0.1 mV or more, an ST depression of 0.1 mV or more, or new appearance of negative U waves is recorded in at least two contiguous leads on the 12-lead ECG during an attack, ECG findings are considered indicative of ischemic change.

(2) Holter Recording
In patients with vasospastic angina, chest pain develops in about 20 to 30% of episodes of ischemic ST change, and many events of coronary spasm are asymptomatic.[69] Because attacks are prevalent between night and early morning at rest, the ischemic ST changes that occur during an attack are often unrecordable except in the hospitalization setting. In such cases, Holter recording is the most useful examination.[70] If ischemia persists for 5 minutes or longer, chest pain is likely to be present; ECG recordings during symptomatic ischemic episodes should be evaluated in detail for characteristics of ST segment levels and the occurrence of arrhythmia. Attention to asymptomatic ST-T changes is also required.

(2) Exercise Test[71–79]
Class I
None
Class IIa
None
Class IIb
Exercise test in patients who are in stable condition and suspected of having vasospastic angina
Class III
Exercise test in patients who are in unstable condition and in whom acute coronary syndrome cannot be ruled out

If an exercise test in the early morning reveals at least one of the following findings, and the findings of ECG and exercise tolerance in the morning differ from those in the daytime, the patient's condition may be vasospastic angina.
(1) Appearance of ST elevation of 0.1 mV or more in at least two contiguous leads during the exercise test
(2) Appearance of ST depression of 0.1 mV or more in at least two contiguous leads during the exercise test
(3) Appearance of negative U waves not observed at rest, during the exercise test

(3) Hyperventilation Test[69,80–91]
Class I
None
Class IIa
Hyperventilation test in patients suspected of having vasospastic angina with a low frequency of attacks
Class IIb
Hyperventilation test in patients suspected of having vasospastic angina with a high frequency of attacks
Class III

Hyperventilation test in patients suspected of having acute coronary syndrome

[Method]

1. It is desirable that the hyperventilation test be conducted at rest in the early morning after an interval of at least 48 hours from administration of vasoactive drugs.
2. Always monitor the 12-lead ECG during the hyperventilation and for 10 minutes after its completion.
3. Measure blood pressure every minute.
4. Place the patient in supine position and obtain the resting 12-lead ECG and blood pressure, and then provide an explanation of hyperventilation. Subsequently, promote vigorous hyperventilation (target: respiratory rate of 25 times/minute or higher) for 6 minutes, to the extent possible for the patient.
5. If the onset of an anginal attack or a significant ST-T change on the ECG is observed during artificial hyperventilation, discontinue it immediately.
6. In the event of an anginal attack, administer a fast-acting nitrate immediately.
7. Evaluation of ST level should be performed at 80 ms after the J point on the ECG.

Criteria for positive ECG Finding of Coronary Spasm on Hyperventilation Test

If at least one of the following findings is obtained, a positive ECG finding of coronary spasm is considered present.

(1) Appearance of ST elevation of 0.1 mV or more in at least two contiguous leads during the hyperventilation test

(2) Appearance of ST depression of 0.1 mV or more in at least two contiguous leads during the hyperventilation test

(3) Appearance of negative U waves not observed at rest, during the hyperventilation test

(4) Evaluation of Vascular Endothelial Function[62,92-101]

Class I
None

Class IIa
None

Class IIb
Vascular endothelial function test in patients suspected of having vasospastic angina

Class III
None

(5) Myocardial Scintigraphy[102-115]

Class I
None

Class IIa
None

Class IIb
1. ^{123}I metaiodobenzylguanidine (^{123}I MIBG) myocardial scintigraphy
2. ^{201}Tl myocardial scintigraphy in combination with hyperventilation test or exercise test
3. ^{123}I β-methyl-branched fatty acid (^{123}I BMIPP) myocardial scintigraphy

Class III
Stress myocardial scintigraphy in patients suspected of having acute coronary syndrome

(6) Others[83-85,91,116-127]

Class I
None

Class IIa
None

Class IIb
Cold pressor test or mental stress test in patients who are in stable condition and suspected of having vasospastic angina

Class III
Cold pressor test or mental stress test in patients suspected of having acute coronary syndrome

2 Invasive Evaluation (Cardiac Catheterization)

A drug-induced coronary spasm provocation test is performed by intracoronary administration of acetylcholine or ergonovine. If increased diagnostic accuracy is desired, a washout period of 2 days or longer for any calcium channel blockers and nitrates should be included whenever possible.

For patients undergoing this examination, adequate informed consent must be obtained before invasive evaluation is performed.

(1) Acetylcholine Provocation Test[16-19,21,56,128,129]

Class I
Acetylcholine provocation test during coronary angiography performed in patients in whom vasospastic angina is suspected based on symptoms, but who have not been diagnosed with coronary spasm by non-invasive evaluation

Class IIa
Acetylcholine provocation test during coronary angiography performed in patients who have been diagnosed with coronary spasm by non-invasive evaluation, and in whom drug treatment is ineffective or insufficiently effective

Class IIb
Acetylcholine provocation test during coronary angiography performed in patients who have been diagnosed with coronary spasm by non-invasive evaluation, and in whom drug treatment has been proven to be effective

Class III
1. Acetylcholine provocation test during coronary angiography performed in patients without symptoms suggestive of vasospastic angina
2. Acetylcholine provocation test during coronary angiography performed in patients who are considered at high risk of suffering a life-threatening complication of induced coronary spasm (eg, patients with left main coronary trunk lesions; those with multivessel coronary lesions, including obstructive lesions; those with severe cardiac dysfunction; and those with untreated congestive heart failure) (however, in cases in which the onset of severe cardiac dysfunction or congestive heart failure may be a consequence of coronary spasm, the criteria for Class IIb apply)
3. Acetylcholine provocation test during emergent coronary angiography performed in patients with acute coronary syndrome

In an acetylcholine- or ergonovine-induced coronary spasm provocation test, coronary spasm is defined as "transient, total, or sub-total occlusion (>90% stenosis) of a coronary artery with signs/symptoms of myocardial ischemia (anginal pain and ischemic ST changes)."