preserved ejection fraction (HFPEF), but also heart failure with reduced ejection fraction (HFREF), is substantially involved.[13,14] Indeed, HFPEF and HFREF respectively account for approximately half of the CHF patients.[15,16]

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We have been conducting a nationwide clinical study supported by the Japanese government on the current status of CHF in Japan with special reference to MetS (MetS-CHF Study). This is the first report of our study, which addresses the prevalence and clinical significance of MetS in Japanese patients with CHF.

Methods

The ethical committees of each institute approved the study protocol and all patients provided written informed consent.

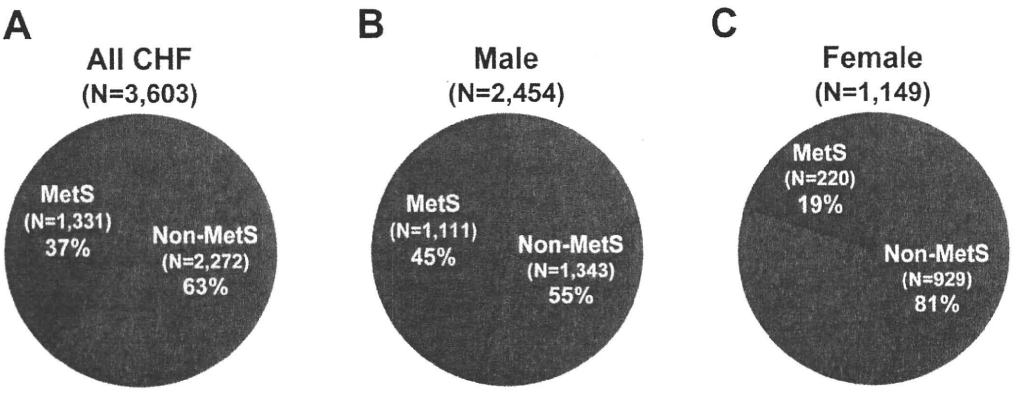
Study Population

Between September 2006 and December 2008 we enrolled 3,603 CHF patients in stages C/D according to the ACC/AHA Guidelines[12] from 6 institutes in Japan. For each patient, we prospectively collected from the participating hospitals

Table 1. Baseline Characteristics of Patients With Symptomatic CHF

	Male (n=2,454)	Female (n=1,149)	P value
Age (years)	67.9±0.2	71.1±0.4	<0.001
Cigarette smoking, n (%)			
Never	811 (48.9%)	856 (79.8%)	<0.001
Former	343 (20.7%)	153 (14.2%)	NS
Current	505 (30.4%)	64 (6.0%)	<0.001
Alcohol intake, n (%)			
Never	722 (30.7%)	760 (80.9%)	<0.001
Former	220 (11.2%)	35 (3.7%)	<0.001
Current	1,027 (52.1%)	144 (15.3%)	<0.001
BMI (kg/m^2)	23.1±0.1	22.1±0.2	<0.001
Waist circumference (cm)	86.7±0.2	81.9±0.4	<0.001
Blood pressure (mmHg)			
Systolic	125.7±0.4	126.3±0.6	NS
Diastolic	72.2±0.3	70.7±0.4	<0.001
Heart rate (beats/min)	71.8±0.3	74.7±0.4	<0.001
NYHA class			
I	490 (20.0%)	133 (11.6%)	<0.001
II	1,683 (68.9%)	814 (70.9%)	NS
III	246 (10.1%)	187 (16.3%)	<0.001
IV	24 (1.0%)	14 (1.2%)	NS
Stage C/D	2,381 (97.4%)/63 (2.6%)	1,113 (97.0%)/35 (3.0%)	NS
LVEF (%)	54.4±0.3	59.5±0.5	<0.001
HFREF (EF<50%)	797 (36.2%)	269 (25.7%)	<0.001
HFPEF (EF≥50%)	1,402 (63.8%)	777 (74.3%)	<0.001
SAS	5.7±0.04	4.6±0.06	<0.001
HT	1,876 (76.4%)	864 (75.2%)	NS
DM or fasting glucose ≥110 mg/dl	1,253 (51.1%)	526 (45.8%)	<0.01
Dyslipidemia	1,754 (71.7%)	816 (71.0%)	NS
IHD	1,264 (51.5%)	352 (30.6%)	<0.001
HHD	230 (9.4%)	126 (11.0%)	NS
CM	508 (20.7%)	216 (18.8%)	NS
VHD	494 (20.1%)	428 (37.2%)	<0.001
CHD	29 (1.2%)	32 (2.8%)	<0.001
Medications			
ACEI/ARB	1,793 (73.1%)	765 (66.6%)	<0.001
β-blocker	1,237 (50.4%)	507 (44.1%)	<0.001
Statin	876 (35.7%)	381 (33.2%)	NS

Values are mean±SEM.
CHF, chronic heart failure; BMI, body mass index; LVEF, left ventricular ejection fraction; HFREF, heart failure with reduced ejection fraction; EF, ejection fraction; HFPEF, heart failure with preserved ejection fraction; SAS, specific activity scale; HT, hypertension; DM, diabetes mellitus; IHD, ischemic heart disease; HHD, hypertensive heart disease; CM, cardiomyopathy; VHD, valvular heart disease; CHD, congenital heart disease; ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin-receptor blocker.



D

	General Population			CHF Patients
	Tanno and Sobetsu Study (Ref. 18)	Hisayama Study (Ref. 19)	Kanazawa Study (Ref. 20)	MetS-CHF Study
Male	18%	21%	16~21%	45%
Female	6%	8%	3~10%	19%

Figure 1. Prevalence of metabolic syndrome (MetS) in patients with chronic heart failure (CHF) was 37% of all patients with CHF (**A**), 46% of male patients (**B**) and 20% of female patients (**C**). Compared with the prevalence in the general population in the Tanno-Sobetsu,[18] Hisayama[19] and Kanazawa[20] studies, the prevalence of MetS was more than double (**D**).

the baseline demographic data, including age, sex, height, weight, waist circumference, coronary risk factors (blood pressure, lipid profile, fasting plasma glucose, smoking status), medications, comorbidities (previous myocardial infarction or stroke, dialysis, and atrial fibrillation) by use of a web data collection system (Tohoku Fujitsu, Sendai, Japan).

Definition of MetS

According to the new definition by the Japanese Committee for the Diagnostic Criteria of MetS in April 2005, we defined MetS as the presence of 2 or more abnormalities in addition to waist circumference (≥85 cm in males and ≥90 cm in females). Other abnormalities examined were dyslipidemia, hypertension, and glucose intolerance/diabetes mellitus. Dyslipidemia was defined as use of lipid-lowering drugs and/or elevated lipid levels (plasma triglycerides ≥150 mg/dl or HDL<40 mg/dl in men or 50 mg/dl in women). Glucose intolerance/diabetes mellitus was defined as use of antidiabetic drugs and/or fasting glucose ≥110 mg/dl. Hypertension was defined as use of antihypertensive drugs and/or systolic blood pressure ≥130 mmHg and/or diastolic blood pressure ≥80 mmHg.

Definition of CHF

In the present study, we included patients with stages C/D CHF defined by the ACC/AHA 2005 Guidelines (ie, they had developed symptoms of HF, at least NYHA class II).[12] According to the ESC 2007 Guideline, we further divided them into 2 groups: HFPEF (LV ejection fraction (EF) ≥50%, n=2,179) and HFREF (LVEF<50%, n=1,066).[17]

Data Collection

Baseline demographic data (age, sex, height, body weight, and waist), CHF stage, medications, including angiotensin-converting enzyme inhibitors (ACEI), angiotensin-receptor blockers (ARB), β-blockers, and statins, risk factors (hypertension, glucose intolerance/diabetes mellitus and dyslipidemia), blood pressure, pulse rate, blood data (lipid profile and glucose), and comorbidities (ischemic heart disease (IHD), hypertensive heart disease, cardiomyopathy, valvular heart disease, and congenital heart disease) were collected from the medical records. LVEF was measured by echocardiography.

Statistical Analysis

Continuous variables are expressed as mean±SEM. Comparisons between 2 groups were conducted with unpaired t-test for continuous variables and chi-test for categorical variables. Statistical analyses were performed using Prism 4 (GraphPad Software, La Jolla, CA, USA). $P<0.05$ was considered to be statistically significant.

Results

Characteristics of CHF Patients

Among the 3,603 consecutive patients with stage C/D CHF, there were 2,454 men (68%, 68±0.2 years) and 1,149 women (32%, 71±0.4 years) (Table 1). In total, 1,331 patients had MetS (37%) and 2,272 did not (63%) (Figure 1A, Table 2). Of the 2,454 male patients with CHF, 1,111 had MetS (45%) and 1,343 did not (55%) (Figure 1B, Table 3), and of the

Table 2. Comparison of Non-MetS and MetS Patients With Symptomatic CHF

	Total		
	Non-MetS (n=2,272)	MetS (n=1,331)	P value
Sex, n (%)			
Male	1,343 (59.1%)	1,111 (83.5%)	<0.001
Female	929 (40.9%)	220 (16.5%)	<0.001
Age (years)	69.7±0.3	67.6±0.3	<0.001
Cigarette smoking, n (%)			
Never	1,129 (63.6%)	538 (56.2%)	<0.001
Former	323 (18.2%)	173 (18.1%)	NS
Current	323 (18.2%)	246 (25.7%)	<0.001
Alcohol intake, n (%)			
Never	1,008 (55.7%)	474 (43.2%)	<0.001
Former	162 (9.0%)	93 (8.5%)	NS
Current	640 (35.3%)	531 (48.3%)	<0.001
BMI (kg/m^2)	21.2±0.1	25.5±0.2	<0.001
Waist circumference (cm)			
Male	81.2±0.2	92.8±0.2	<0.001
Female	77.9±0.3	97.1±0.5	<0.001
Blood pressure (mmHg)			
Systolic	123.6±0.4	129.8±0.5	<0.001
Diastolic	70.3±0.3	74.2±0.3	<0.001
Heart rate (beats/min)	72.8±0.3	72.6±0.4	NS
NYHA class			
I	354 (15.6%)	269 (20.3%)	<0.001
II	1,576 (69.6%)	921 (69.5%)	NS
III	303 (13.4%)	130 (9.8%)	<0.001
IV	32 (1.4%)	6 (0.4%)	<0.001
Stage C/D	2,193 (96.7%)/75 (3.3%)	1,301 (98.3%)/23 (1.7%)	<0.01
LVEF (%)	55.7±0.4	56.7±0.4	NS
HFREF (EF<50%)	700 (34.0%)	366 (30.8%)	NS
HFPEF (EF≥50%)	1,357 (66.0%)	822 (69.2%)	NS
SAS	5.2±0.05	5.6±0.06	<0.001
HT	1,525 (67.1%)	1,215 (91.3%)	<0.001
DM or fasting glucose≥110 mg/dl	890 (39.2%)	889 (66.8%)	<0.001
Dyslipidemia	1,402 (61.7%)	1,168 (87.8%)	<0.001
IHD	882 (38.8%)	734 (55.1%)	<0.001
HHD	192 (8.5%)	164 (12.3%)	<0.001
CM	477 (21.0%)	247 (18.6%)	NS
VHD	714 (31.4%)	208 (15.6%)	<0.001
CHD	49 (2.2%)	12 (0.9%)	<0.01
Medications			
ACEI/ARB	1,534 (67.5%)	1,024 (76.9%)	<0.001
β-blocker	1,058 (46.6%)	686 (51.5%)	<0.01
Statin	638 (28.1%)	619 (46.6%)	<0.001

Values are mean±SEM.
MetS, metabolic syndrome. Other abbreviations see in Table 1.

1,149 female patients with CHF, 220 had MetS (19%) and 929 did not (81%) (Figure 1C, Table 3). The prevalence of MetS in the general Japanese population has been previously reported as approximately 20% in men and approximately 10% in women in the Tanno-Sobetsu Study, the Hisayama Study (males 58±11 years, females 59±11 years), and the Kanazawa Study (males 68±8 years, females 66±9 years),[7,18–20] so our results show a prevalence of MetS in Japanese CHF patients as more than double that of the general population (Figure 1D).

As shown in Table 1, the present stage C/D CHF patients were characterized by a higher prevalence of hypertension and dyslipidemia, followed by glucose intolerance/diabetes mellitus, in both sexes. Furthermore, the male CHF patients

Table 3. Comparison of Non-MetS and MetS Patients With Symptomatic CHF

	Male			Female		
	Non-MetS (n=1,343)	MetS (n=1,111)	P value	Non-MetS (n=929)	MetS (n=220)	P value
Age (years)	68.9±0.3	66.6±0.3	<0.001	70.8±0.4	72.6±0.7	<0.05
Cigarette smoking, n (%)						
Never	441 (48.6%)	370 (49.3%)	NS	688 (79.4%)	168 (81.6%)	NS
Former	195 (21.4%)	148 (19.7%)	NS	128 (14.8%)	25 (12.1%)	NS
Current	272 (30.0%)	233 (31.0%)	NS	51 (5.8%)	13 (6.3%)	NS
Alcohol intake, n (%)						
Never	402 (37.9%)	320 (35.3%)	NS	606 (81.0%)	154 (80.6%)	NS
Former	131 (12.3%)	89 (9.8%)	NS	31 (4.2%)	4 (2.1%)	NS
Current	529 (49.8%)	498 (54.9%)	NS	111 (14.8%)	33 (17.3%)	NS
BMI (kg/m^2)	21.3±0.2	25.3±0.2	<0.001	21.1±0.2	26.5±0.5	<0.001
Blood pressure (mmHg)						
Systolic	122.6±0.6	129.4±0.5	<0.001	125.1±0.7	131.6±1.4	<0.001
Diastolic	70.2±0.3	74.6±0.4	<0.001	70.4±0.04	72.2±0.9	NS
Heart rate (beats/min)	71.5±0.4	72.2±0.4	NS	74.7±0.6	74.7±1.0	NS
NYHA class						
I	240 (18.0%)	250 (22.6%)	<0.01	114 (12.3%)	19 (8.6%)	NS
II	929 (69.5%)	754 (68.2%)	NS	647 (69.7%)	167 (75.9%)	NS
III	149 (11.1%)	97 (8.8%)	NS	154 (16.6%)	33 (15.0%)	NS
IV	19 (1.4%)	5 (0.4%)	<0.001	13 (1.4%)	1 (0.5%)	NS
Stage C/D	1,296 (96.7%)/ 44 (3.7%)	1,085 (98.3%)/ 19 (1.7%)	<0.05	897 (96.7%)/ 31 (3.3%)	216 (98.2%)/ 4 (1.8%)	NS
LVEF (%)	53.3±0.5	55.8±0.5	<0.001	59±0.5	61.4±1.0	NS
HFREF (EF<50%)	468 (38.7%)	329 (33.2%)	<0.01	232 (27.3%)	37 (18.8%)	<0.05
HFPEF (EF≥50%)	740 (61.3%)	662 (66.8%)	<0.01	617 (72.7%)	160 (81.2%)	<0.05
SAS	5.6±0.06	5.8±0.06	<0.05	4.6±0.07	4.5±0.1	<0.001
HT	870 (64.8%)	1,006 (90.5%)	<0.001	655 (70.5%)	209 (95.0%)	<0.001
DM or fasting glucose≥110 mg/dl	506 (37.7%)	747 (67.2%)	<0.001	384 (41.3%)	142 (64.5%)	<0.001
Dyslipidemia	787 (58.6%)	967 (87.0%)	<0.001	615 (66.2%)	201 (87.3%)	<0.001
IHD	632 (47.1%)	632 (56.9%)	<0.001	250 (27.0%)	102 (46.4%)	<0.001
HHD	104 (7.7%)	126 (11.3%)	<0.01	88 (9.5%)	38 (17.3%)	0.001
CM	294 (21.9%)	214 (19.3%)	NS	183 (19.8%)	33 (15.0%)	NS
VHD	332 (24.7%)	162 (14.6%)	<0.001	382 (41.3%)	46 (20.9%)	<0.001
CHD	19 (1.4%)	10 (0.9%)	NS	30 (3.2%)	2 (1%)	NS
Medications						
ACEI/ARB	935 (69.6%)	858 (77.2%)	<0.001	599 (64.5%)	166 (75.5%)	<0.01
β-blocker	666 (49.6%)	571 (51.4%)	NS	392 (42.2%)	115 (52.3%)	<0.01
Statin	364 (27.1%)	512 (46.1%)	<0.001	274 (29.5%)	107 (48.6%)	<0.001

Values are mean±SEM.
Abbreviations see in Tables 1,2.

were characterized by higher prevalence of larger body mass index, glucose intolerance/diabetes mellitus, and IHD, whereas the female patients were in a higher NYHA class, had lower exercise tolerance, and higher prevalence of both preserved LVEF and valvular heart disease.

MetS in CHF

In both male and female patients with CHF, those with MetS were characterized by younger age, higher prevalence of current smoking and drinking, IHD, and hypertensive heart disease, lower NYHA class, better exercise tolerance, and more likelihood of taking medications such as ACEI/ARB, β-blockers or statins (Tables 2,3, Figure 2). The prevalence of HFPEF was significantly higher in the MetS group compared with the non-MetS group (Table 3, Figure 3).

When compared with the patients with HFREF, those with HFPEF were characterized by higher prevalence of elderly and female patients, obesity, hypertensive and valvular heart disease, and less likelihood of taking medications such as ACEI/ARB, β-blockers or statins (Table 4).

Metabolic Components in CHF

In the present study, the contribution of single or combined metabolic components was observed in both the ischemic

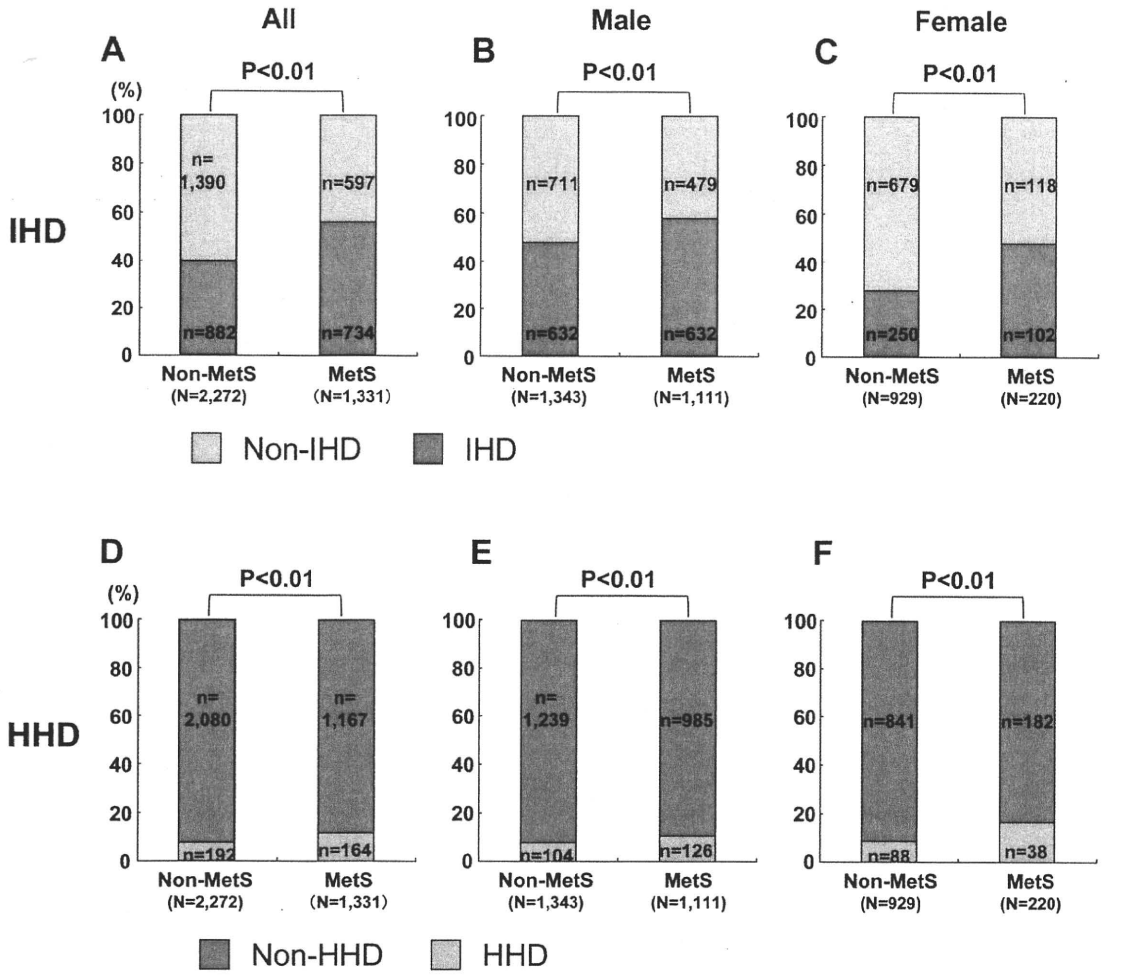


Figure 2. Effect of metabolic syndrome (MetS) on ischemic heart disease (IHD) and hypertensive heart disease (HHD). IHD was significantly more prevalent in CHF patients with MetS than in those without it for all patients (**A**), and in male (**B**) or female patients (**C**). Similarly, for HHD for all patients (**D**), and male (**E**) and female patients (**F**).

and non-ischemic CHF patients (Figure 4A). Although the prevalence of ischemic CHF was significantly higher in most of the subgroups with more than 3 metabolic components, the contribution of other single or combined metabolic components was either comparable between the 2 groups or stronger in the non-ischemic CHF group (Figure 4A). Although the prevalence of combined metabolic components varied, these components were comparably associated with both HFPEF and HFREF (Figure 4B).

Discussion

The novel findings of the present study are that (a) the prevalence of MetS in CHF was more than double that of the general Japanese population, (b) MetS was associated with ischemic or hypertensive heart disease-related heart failure, (c) HFPEF was characterized by a higher prevalence of elderly and female patients with MetS, and (d) the prevalence of the metabolic components was comparable between the ischemic and non-ischemic CHF patients. To the best of our knowledge, this is the first study to provide evidence for a relationship between MetS and CHF.

Prevalence of MetS in CHF

It has been reported that the prevalence of MetS in the general Japanese population is 10–20% in men and 2–8% in women, as defined by the current Japanese criteria.[7,18,19] In contrast, the present study demonstrated a prevalence of MetS (45% in men and 19% in women) that is more than 2-fold that of the general population, suggesting that the presence of MetS is an important therapeutic target of CHF treatment. It is conceivable that the increased prevalence of MetS in CHF patients is both the cause and the result of CHF, as activation of both the sympathetic nervous system and renin–angiotensin system causes the metabolic components.[21] In order to address this important issue, we are now performing a cohort study in which we follow-up MetS patients without CHF to examine the development of CHF in them.

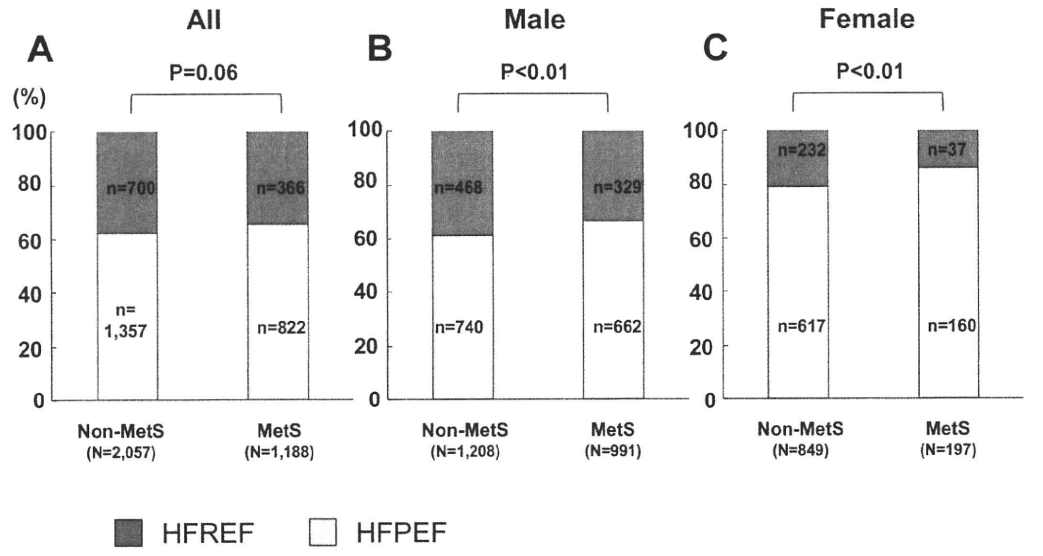


Figure 3. Effect of metabolic syndrome (MetS) on left ventricular ejection fraction. The prevalence of heart failure with preserved ejection fraction (HFPEF) was significantly greater for males (B) and females (C) with MetS than in those without MetS and tended to be so for all patients with chronic heart failure (A). HFREF, heart failure with reduced ejection fraction.

Role of MetS in Ischemic and Hypertensive Heart Disease

MetS has been identified as a risk and prognostic factor for IHD and stroke.[8,22,23] In the present study, MetS was highly associated with IHD in both male and female patients with CHF. Thus, the prevention of IHD is extremely important for preventing the development of CHF, both by life-style modification and the use of anti-atherosclerotic drugs in order to achieve stabilization and regression of systemic atherosclerosis. Furthermore, because hypertension is associated with obesity,[24] it is also important to treat obesity for blood pressure control in order to prevent the development of hypertensive heart disease.

Comparison of HFPEF and HFREF

It has been demonstrated that heart failure can also occur in patients with preserved LVEF, which is often observed in hypertensive heart disease mainly caused by LV diastolic dysfunction.[14] It is now widely accepted that HFPEF is a major cardiovascular disorder with poor prognosis, accounting for approximately 50% of patients with heart failure symptoms,[15,16] and our study demonstrated that 67% of CHF patients had HFPEF (Table 4). The present results also indicate the different clinical characteristics of HFPEF and HFREF patients, and the former were characterized by a higher prevalence of elderly and female patients, obesity, and hypertensive and valvular heart disease. Although it has been previously demonstrated that the major determinants of diastolic dysfunction are enhanced myocardial stiffness and impaired relaxation capacity,[25] further studies are needed to clarify the association between these clinical factors and LV dysfunction.

Metabolic Components in Ischemic and Non-Ischemic CHF

In the present study, among the metabolic components in the CHF patients, the prevalence of both hypertension and dyslipidemia was higher, followed by glucose intolerance/diabetes mellitus, probably because of environmental and genetic factors. In order to prevent the development of CHF, all components of MetS should be controlled (ie, blood pressure by anti-hypertensive drugs, lipid-lowering by HMG-CoA reductase inhibitors, and glucose control by diet therapy, exercise and antidiabetic drugs), which is known to ameliorate vascular function and stabilize atheroma.[26–33] In contrast, smoking and alcohol intake may not be highly related to the development of CHF compared with hypertension or dyslipidemia, so smoking cessation and moderate alcohol intake are recommended in the early stage of CHF.[9]

In the present study, MetS was related to the development of HFPEF (LVEF≥50%) in both male and female patients with CHF. Although the precise mechanisms are unknown, coronary microvascular dysfunction with preserved systolic function might be linked to this phenomenon.[34,35]

The present study also demonstrated that there are single or combined metabolic components in both non-ischemic CHF and ischemic CHF patients, a consistent finding with a previous report regarding the lipid levels and heart failure incidence in Caucasians.[36] Therefore, these metabolic components should be regarded as important therapeutic targets for CHF caused by both ischemic and non-IHD.

Study Limitations

First, although we were able to collect the data for a relatively large number of CHF patients, their prognoses need to be elucidated. As we are currently performing a follow-up study for them, we will report the results separately in the future. Second, we used the 2005 definition of the Japanese Committee for the Diagnostic Criteria of MetS, so we were unable to compare the present data with that of non-Japanese studies. We plan to use other diagnostic criteria, such as the National Cholesterol Education Program-Adult Treatment

Table 4. Comparison of HFPEF and HFREF Patients With Symptomatic CHF

	Total		
	HFPEF (n=2,179)	HFREF (n=1,066)	P value
Sex, n (%)			
Male	1,402 (64.3%)	797 (74.8%)	<0.001
Female	777 (35.7%)	269 (25.2%)	<0.001
Age (years)	69.6±0.3	67.7±0.4	<0.001
Non-MetS	1,357 (62.3%)	700 (65.7%)	0.06
MetS	822 (37.7%)	366 (34.3%)	0.06
Cigarette smoking, n (%)			
Never	453 (41.2%)	470 (60.4%)	<0.01
Former	307 (27.9%)	133 (17.1%)	<0.01
Current	339 (30.8%)	175 (22.5%)	<0.01
Alcohol intake, n (%)			
Never	925 (52.2%)	435 (49.7%)	<0.001
Former	149 (8.4%)	85 (9.7%)	<0.001
Current	699 (39.4%)	356 (40.6%)	<0.001
BMI (kg/m^2)	23.1±0.1	22.4±0.2	<0.01
Waist circumference (cm)			
Male	86.9±0.3	85.9±0.3	<0.001
Female	82.1±0.4	80.9±0.8	<0.001
Blood pressure (mmHg)			
Systolic	128.1±0.4	120.7±0.6	<0.001
Diastolic	72.2±0.3	70.5±0.4	<0.001
Heart rate (beats/min)	72.3±0.3	73.8±0.5	<0.05
NYHA class			
I	429 (19.7%)	134 (12.6%)	<0.001
II	1,510 (69.5%)	743 (69.9%)	NS
III	215 (9.9%)	173 (16.3%)	<0.001
IV	19 (0.9%)	13 (1.2%)	NS
Stage C/D	2,125 (97.7%)/49 (2.3%)	1,027 (96.5%)/37 (3.5%)	<0.05
LVEF (%)	65.3±0.2	37.2±0.3	<0.001
SAS	5.4±0.05	5.0±0.06	<0.001
HT	1,725 (53.2%)	729 (68.4%)	<0.001
DM or fasting glucose≥110mg/dl	1,042 (47.8%)	554 (52.0%)	<0.05
Dyslipidemia	1,499 (68.8%)	802 (75.2%)	<0.001
IHD	894 (41.0%)	501 (47.0%)	<0.001
HHD	258 (11.8%)	73 (6.8%)	<0.001
CM	319 (14.6%)	365 (34.2%)	<0.001
VHD	695 (31.9%)	169 (15.9%)	<0.001
CHD	48 (2.2%)	6 (0.6%)	<0.01
Medications			
ACEI/ARB	1,483 (68.1%)	835 (78.3%)	<0.001
β-blocker	880 (40.9%)	711 (66.7%)	<0.001
Statin	702 (32.2%)	403 (37.8%)	<0.01

Values are mean±SEM.
Abbreviations see in Tables 1,2.

Panel III (NCEP/ATPIII),[37] American Heart Association/National Heart, Lung, and Blood Institute (AHA/NHLBI),[38] and International Diabetes Federation (IDF),[39] in future analyses. Third, although MetS is the association and clustering of metabolic components, we were unable to exclude CHF patients complicated by severe hypertension, severe dyslipidemia, or severe diabetes mellitus. This issue also remains to be examined in future studies. Last, the present study lacks an appropriate control group in the same population, which why we used the data from the Kanazawa Study of the Japanese general population in 2007 that demonstrated a prevalence of MetS of 16–21% in 50- to 80-year-old males and in females, prevalence of 3% in the 50s, 5% in the 60s, 8% in the 70s, and 10% in the 80s.[20]

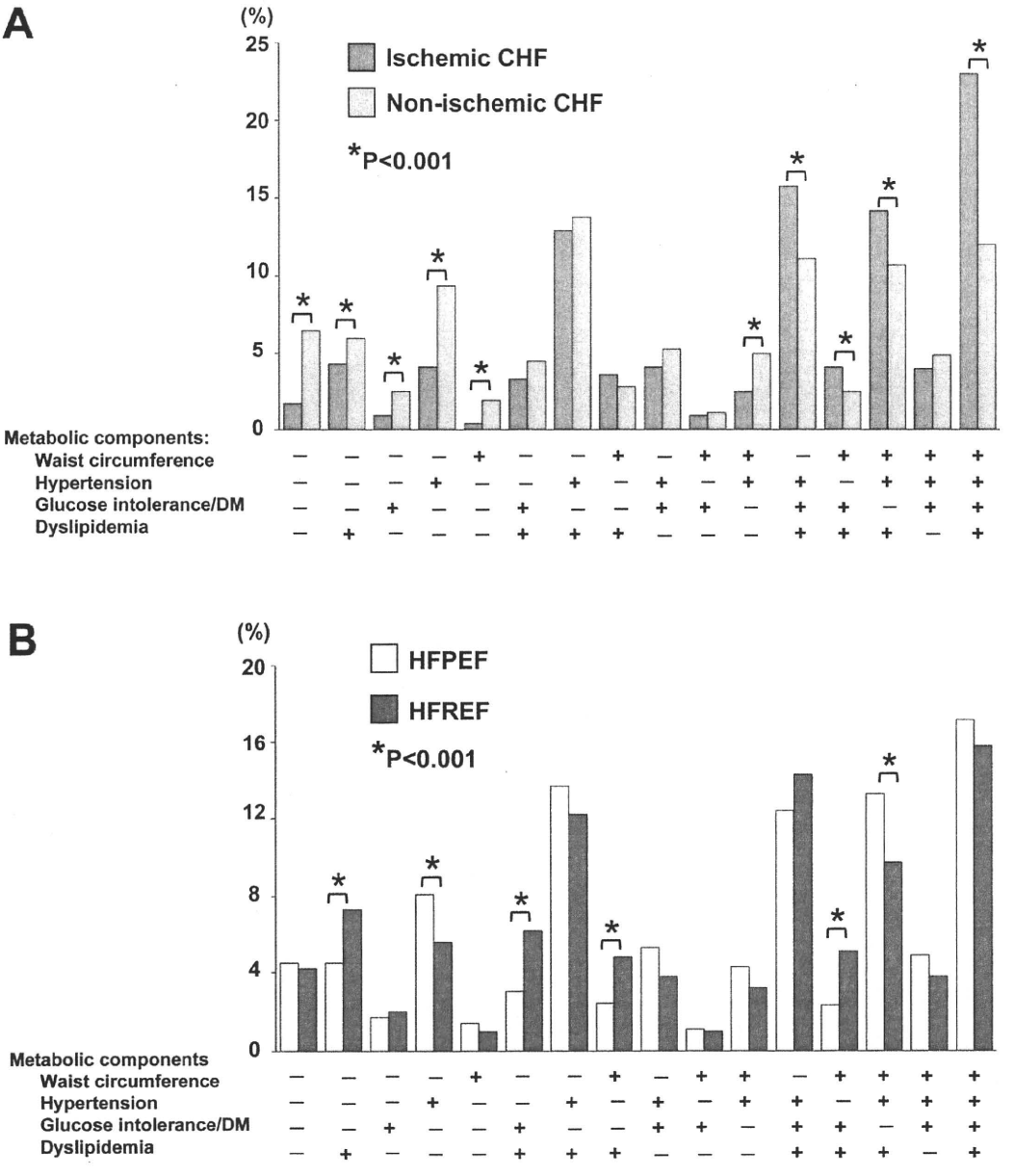


Figure 4. Accumulation of metabolic components in patients with chronic heart failure (CHF). (A) When the number of metabolic components was greater than 3, their contribution to ischemic heart failure was greater, whereas the contribution of other single or combined metabolic components was comparable between the 2 groups or stronger in the non-ischemic heart failure group. (B) Although the prevalence of combined metabolic components varied, the components were equally associated with both heart failure with preserved ejection fraction (HFPEF) and heart failure with reduced ejection fraction (HFREF). DM, diabetes mellitus.

Conclusion

We found that the prevalence of MetS in CHF patients was more than double compared with the general population in Japan, with a greater involvement of ischemic or hypertensive heart disease and a higher prevalence in elderly and female patients. Because the metabolic components might have a substantial effect on the development of both ischemic and non-ischemic CHF, MetS should be regarded as a new therapeutic target for this disorder.

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Disclosures

None.

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The First Clinical Pilot Study of Intravenous Adrenomedullin Administration in Patients With Acute Myocardial Infarction

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Abstract: Adrenomedullin (AM) is a 52-amino-acid vasodilator peptide that was originally isolated from human pheochromocytoma. In the previous experimental study with rat ischemia/reperfusion model, AM reduced infarct size and inhibited myocyte apoptosis. AM also suppressed the production of oxygen-free radicals. The present study was designed to evaluate the feasibility of intravenous administration of AM in patients with acute myocardial infarction. We studied 10 patients with first acute myocardial infarction [male to female ratio: 9 to 1, age: 65 ± 9 (mean ± SD) years, peak creatine phosphokinase level: 4215 ± 1933 (SD) U/L], who were hospitalized within 12 hours of symptom onset. Proceeding reperfusion therapy, AM infusion was initiated and continued at concentration of 0.0125–0.025 $\mu g \cdot kg^{-1} \cdot min^{-1}$ for 12 hours. Follow-up coronary angiography and left ventriculography were performed at 3 months. Cardiac magnetic resonance was examined at 1 month and 3 months after AM therapy. During infusion of AM, hemodynamics kept stable except 2 patients. Wall motion index in the infarct area at 3 months was significantly improved compared with that at baseline, and infarct size evaluated by cardiac magnetic resonance was significantly decreased at 3 months. In conclusion, intravenous administration of AM, which possesses a variety of potential cardiovascular protective actions, can be adjunctive to percutaneous coronary intervention.

Key Words: acute myocardial infarction, adrenomedullin, cardioprotective therapy, reperfusion injury

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INTRODUCTION

Reperfusion therapy by using percutaneous coronary intervention (PCI) reduces infarct size, improves left ventricular function, and leads to better clinical outcomes in patients with acute myocardial infarction (AMI).[1] However, reperfusion therapy could also elicit adverse reactions that may limit its beneficial action, leading to irreversible cardiac damage.[2,3] Although many basic and clinical studies were performed to reveal the effective adjunctive therapy for the reduction or prevention of those reactions, few agents are available in the clinical settings.[4–6] Therefore, a novel cardioprotective agent is emerging to prevent ischemia/reperfusion injury in patients with AMI.

Adrenomedullin (AM) is a 52-amino-acid vasodilator peptide that was originally isolated from human pheochromocytoma.[7] In the previous experimental study with rat ischemia/reperfusion model, AM attenuated myocardial ischemia/reperfusion injury, reduced infarct size, and inhibited myocyte apoptosis via a PIK3/Akt-dependent pathway, which leads to the prevention of myocardial injury after transient ischemia.[8] Moreover, AM also increases nitric oxide bioactivity and suppresses the production of reactive oxygen species,[9] which is one of important factor to develop ischemia/reperfusion injury.[10] This pilot study was therefore designed to evaluate the clinical feasibility of intravenous administration of AM in patients with AMI.

PATIENTS AND METHODS

Study Subjects

The present study is a single-center, prospective, nonrandomized trial. Eligibility criteria were age between 20 and 79 years, chest pain for more than 30 minutes, at least 0.1 mV of ST segment elevation in 2 adjacent electrocardiogram (ECG) leads, admission to hospital within 12 hours of the onset of symptoms, and first episode of AMI. Exclusion criteria were a history of myocardial infarction, left main trunk stenosis, cardiogenic shock, severe liver or kidney dysfunction or both, suspected aortic dissection, previous coronary artery bypass grafting, and a history of drug allergy. This trial was reviewed and approved by the ethics review committee, and written informed consent was obtained from all patients.

Study Protocol

After written informed consent was obtained, intravenous administration of AM was initiated 72 minutes (median; 39–145) before the reperfusion therapy and continued for 12 hours (Fig. 1). The time interval from the onset of AMI to the initiation of AM was 303 (median: 132–612) minutes. In the first 6 patients, AM was used at the dose of 0.025 μg·(kg body weight)$^{-1}$·min^{-1}. Of these patients, severe hypotension occurred at 2 hours after AM infusion therapy in one patient with inferior and right ventricular infarction. Therefore, in the next 4 patients, the administration of AM was changed as follows: AM was administrated intravenously for the first 3 hours at the dose of 0.025 μg·(kg body weight)$^{-1}$·min^{-1}, which was reduced to the dose of 0.0125 μg·(kg body weight)$^{-1}$·min^{-1} for the following 9 hours. When blood pressure was below 90 mm Hg during AM administration at 0.025 μg·(kg body weight)$^{-1}$·min^{-1}, the dose was reduced to 0.0125 μg·(kg body weight)$^{-1}$·min^{-1}. If blood pressure did not increase over 90 mm Hg even after decreasing dose, the administration was discontinued. Emergent coronary angiography followed by PCI were performed to restore Thrombolysis in Myocardial Infarction (TIMI) III grade flow in the infarct-related artery by using bare metal stent. After PCI, left ventriculography (LVG) was performed to evaluate left ventricular function. By using a 7.5-F Swan-Ganz catheter (TOO21H-7.5F; Baxter, Co), hemodynamic parameters, including heart rate (HR), mean arterial pressure, mean pulmonary arterial pressure (mPA), pulmonary capillary wedge pressure (PCWP), and cardiac index (CI), were continuously monitored for 24 hours after PCI. Cardiac magnetic resonance (CMR) was performed to assess risk area at 1 month when the effect of the infarction-related myocardial edema was likely to be minimized.[11,12] Coronary angiography, LVG, and CMR were reevaluated 3 months after AM therapy. The study protocol did not restrict any other therapeutic strategies including the reperfusion method such as PCI or thrombolytic therapy. Other cardiovascular medications were allowed at the doctor's discretion.

Preparation of Human AM

Human AM was obtained from Peptide Institute, Inc, Osaka, Japan. The homogeneity of human AM was confirmed using reverse-phase high-performance liquid chromatography and amino acid analysis. AM was dissolved in saline with 4% D-mannitol and sterilized through a 0.22-μm filter (Millipore, Co, Billerica, MA). Then, randomly selected vials were submitted for sterility and pyrogen testing. The chemical nature and content of the human AM in the vials were verified using high-performance liquid chromatography and radioimmunoassay. Mature AM level was measured using immunoradiometric assays with a specific kit for this marker (Shionogi, Co, Ltd, Osaka, Japan).

Coronary Angiography and PCI

Selective coronary angiography was preformed in multiple projections after administration of intracoronary nitroglycerin (0.125–0.25 mg). After the culprit lesion was revealed by coronary angiography, emergent PCI was performed. All procedural decisions, including device selection and intravascular ultrasound, were made at the discretion of the individual PCI operator. Intravenous heparin (5000 IU) and intracoronary nitroglycerin (0.5 mg) were administered before the PCI. Procedural success was defined as the residual stenosis <50% without major complications. Grading of collateral circulation was as follows: none (no visible filling of any collateral channels), poor (filling of side branches only), fair (partial filling of the epicardial vessel), and good (complete filling of the epicardial vessel).[10] Slow flow was defined as TIMI grade II flow, and no reflow was defined as TIMI grade 0 or I flow in the distal infarct-related artery despite of the absence of an occlusion or dissection at the treatment site. Dual antiplatelet therapy (aspirin 300 mg and ticlopidine 200 mg) was instituted in all patients treated with bare metals stent for at least 2 weeks.

Assessment of Infarct Size and Reperfusion Quality

Blood samples were obtained on admission and at 3-hour intervals. Creatine phosphokinase (CPK) and CPK-MB levels were measured at 3-hour intervals until CPK and CPK-MB levels peaked, and those values were used as the enzymatic marker of infarct size. End-diastolic end-systolic volumes and ejection fraction were measured by the area-length method in LVG. Centerline chord motion analysis was used to assess quantitatively regional left ventricular wall motion (QLV-CMS version 5.0; Medis Medical Imaging Systems).[13] In brief, the centerline is constructed midway between the end-diastolic and end-systolic left ventricular

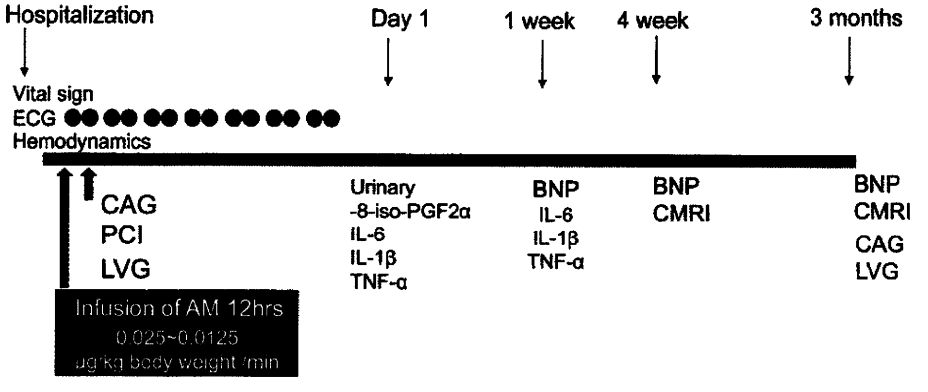

FIGURE 1. Study protocol. CAG, coronary angiography; 8-iso-PGF2α, 8-iso-prostaglandin F2α.

contours in the right anterior oblique projection. Wall motion is measured along 100 chords constructed perpendicular to the centerline. A fractional shortening at each chord was calculated. In the present study, wall motion index was defined as the averaged fractional shortening in the infracted and noninfarcted area. Reperfusion quality (eg, coronary microcirculation) was assessed by ST segment resolution and myocardial blush grade. Complete ST resolution was defined as a reduction of at least 50% in ST segment elevation on ECGs obtained 1 hour after reperfusion compared with the initial value.[14] Myocardial blush score was determined by 2 observers, who were unaware of the clinical and angiographic findings, using a previously reported grading scale.[15]

Cardiac Magnetic Resonance Protocol

CMR procedure was performed with a 1.5T clinical scanner (Sonata; Siemens, Erlangen, Germany) using a 4-element phased array cardiac receiver coil. The ECG-gated images were acquired during repeated breath holds of varying duration depending on HR. Cine images using a segmented true fast imaging with steady-state precession sequence (6-mm slice thickness) were obtained in multiple short-axis views for every 10 mm covering the whole right ventricle and left ventricle. Black blood spin echo images were obtained using half-Fourier single-shot turbo spin echo, with echo time of 24 milliseconds for T1-weighted imaging and echo time of 82 milliseconds for T2-weighted imaging. In both types of imaging, matrix = 256 × 151 and voxel = 1.3 × 2.2 × 6.0 mm^3. Fat suppression was employed in T2-weighted imaging. Late gadolinium enhancement (LGE) was performed with 0.15 mM/kg of gadolinium diethyltriaminepentaacetic acid (Magnevist; Byer-Schering AG, Berlin, Germany). A multislice, breath-hold, inversion-recovery true fast imaging with steady-state precession sequence for LGE was performed sequentially before contrast injection and 2, 5, 10, and 20 minutes after injection [repetition time/echo time = 1.74/3.48 milliseconds, flip angle 60 degree, matrix = 256 × 172, typical voxel size = 1.3 × 2.0 × 8.0 mm^3, inversion time fixed at 300 milliseconds].

CMR Image Analysis

Global ventricular volumes and function were calculated from the short-axis cine images using a disk summation method (Simpson rule), with integration over image slices using the ARGUS system (Siemens, Erlangen, Germany). Measurements of biventricular volumes, end-diastolic volume index (EDVI), end-systolic volume index (ESVI), and ejection fraction were performed in all patients. LGE in both right and left ventricles was interpreted on images 10 minutes after contrast injection by 2 experienced cardiovascular radiologists unaware of the clinical findings. LGE was assigned to be positive when a segment had image intensity 2 or more SDs above the mean of remote normal myocardium.[16] To quantify the infarct size, hyperenhanced areas were manually traced on short-axis delayed hyperenhancement images, except for the most basal and apical slices, and measured by 2 experienced cardiologist and radiologist. Total mass was calculated as follows: myocardial area × sliced thickness × myocardial specific gravity of 1.05. Relative infarct LV mass (%) was defined as sum of the infracted LV area/total LV area.

Biochemical Measurements

Urinary 8-iso-PGF2α for 24 hours after admission was measured. The plasma levels of tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β were measured before infusion of AM and at 24 hours and 3 months after infusion of AM. Plasma brain natriuretic peptide (BNP) was measured at 1 week, 1 month, and 3 months after infusion of AM. BNP was measured by immunoradiometric assay using specific kit (Shionogi, Co, Ltd). Urinary 8-iso-PGF2α was measured using commercially available enzyme-linked immunosorbent assay kits (Cayman, Ann Arbor, MI). IL-6 was measured by a high-sensitivity enzyme immunoassay (R&D Systems, Inc, Minneapolis, MN). IL-1β was measured using a highly sensitive immunoassay (Quantikine HS; R&D systems). TNF-α was measured by using enzyme-linked immunosorbent assay (R&D Systems, Inc).

Statistical Analysis

Continuous variables are expressed as mean ± SD. Comparisons of clinical parameters between admission and 3 months after infusion of AM were performed by Fisher exact test or unpaired Student test. Repeated-measures analysis of variance was used for comparison of the serial data followed by the multiple comparison test. $P < 0.05$ was considered statistically significant.

RESULTS

Patient Characteristics and Reperfusion Therapy

Between October 2003 and February 2006, 10 patients with the first AMI were enrolled in this study (Table 1). The procedural data of PCI were summarized in Table 2. Intravenous thrombolysis with monteplase [24000 IU/(kg body weight)] was performed before PCI in one patient. Finally, all patients underwent bare metal stent implantation after coronary thrombectomy. Reperfusion arrhythmia was not induced during PCI procedure except accelerated idioventricular rhythm in one patient. Moreover, no patients exhibited additional ST reelevation, reflow, or slow flow phenomenon. Complete ST resolution and blush score more than 2 were in 8 patients. The incidence of new Q-wave appearance in ECG was 70%. Urinary 8-iso-PGF2α level for 24 hours was 145.3 ± 84.6 pmol/mmol creatinine.

Hemodynamic Changes in Response to AM Administration

In the first 6 patients treated at the AM dose of 0.025 μg·(kg body weight)$^{-1}$·min^{-1}, the plasma AM concentration increased to 34.62 fmol/mL at 3 hours and then kept at similar level until its discontinuation at 12 hours. In the reaming 4 patients treated at the AM dose of 0.0125 μg·(kg body weight)$^{-1}$·min^{-1}, the plasma AM concentration increased to 33.77 fmol/mL at 3 hours, decreased to 10.45 fmol/mL at 6 hours, and then kept at similar level until its discontinuation at 12 hours (Fig. 2).

The changes in blood pressure and HR during AM administration were summarized in Figure 3. Although HR did

TABLE 1. Baseline Clinical Characteristics (n = 10)

Age, years (±SD)	65 ± 9
Male, n (%)	9 (90)
Diabetes mellitus, n (%)	5 (50)
Hypertension, n (%)	8 (80)
Hyperlipidemia, n (%)	6 (60)
Family history, n (%)	2 (20)
Current smoker, n (%)	7 (70)
Elapsed time from the onset, min (±SD)	262 ± 150
Infarct site	
Anterior	4
Inferior	5
Posterior	1
Culprit lesion	
LAD	4
LCX	1
RCA	5
Collaterals	
None	5
Poor	1
Fair	4
Good	0
Peak CPK, IU/L (±SD)	4215 ± 1933
Peak creatine kinase MB, IU/L (±SD)	487 ± 255

LAD, left anterior descending artery; LCX, left circumflex artery; RCA, right coronary artery; CPK, creatine phosphokinase.

TABLE 2. PCI Procedural Data (n = 10)

Intravenous thrombolysis, n (%)	1 (10)
Coronary thrombectomy, n (%)	10 (100)
Bare metal stent, n (%)	10 (100)
Pre-TIMI grade flow, n (%)	
0	8 (80)
I	1 (10)
II	0 (0)
III	1 (10)
Final TIMI grade flow, n (%)	
0	0 (0)
I	0 (0)
II	0 (0)
III	10 (100)
Reperfusion arrhythmia, n (%)	
AIVR/PVC	0 (0)
VT/VF	0 (0)
Bradycardia/AV block	0 (0)
Complete ST resolution, n (%)	8 (80)
ST reelevation, n (%)	0 (0)
Blush grade score, n (%)	
≤1	2 (20)
≥2	8 (80)
Slow flow, n (%)	0 (0)
No reflow, n (%)	0 (0)

AIVR, accelerated idioventricular rhythm; AV block, atrioventricular block; PVC, premature ventricular contraction; VF, ventricular fibrillation; VT, ventricular tachycardia.

not change, both systolic and diastolic blood pressure decreased to the significant level 120 minutes after AM administration. This blood pressure–lowering effects of AM continued until 12 hours but disappeared 6 hours after discontinuation of AM (at 18-hour time point). The changes in hemodynamic parameters were similar with those in blood pressure. As shown in Figure 4, during AM administration, PCWP and mPA were significantly decreased, which is accompanied with the 38% increase in CI.

Adverse Cardiac Events

Two adverse cardiac events occurred in acute phase. In a patient with inferior and right ventricular infarction, blood pressure decreased to 56/38 mm Hg 2 hours after 0.025 $\mu g \cdot (kg\ body\ weight)^{-1} \cdot min^{-1}$ AM administration but returned to 106/68 mm Hg soon after its discontinuation. In another patient with broad anterior myocardial infarction, hypotension (78/54 mm Hg) developed 3 hours after 0.0125 $\mu g \cdot (kg\ body\ weight)^{-1} \cdot min^{-1}$ AM administration and did not improve even after its discontinuation. Echocardiography revealed the complication of ventricular septal and free-wall perforation with pericardial effusion. Despite of mechanical circulatory support, this particular patient died of cardiogenic shock due to cardiac rupture 42 hours after the onset of AMI. The serum AM level at 3 hours was 13.16 pmol/L in this particular patient. The autopsy study revealed acute hemorrhagic infarction of broad anteroseptal wall and confirmed dissecting rupture of ventricular septum, anterior papillary muscle, and apex.

Left Ventricular Assessment by LVG, CMR, and Biochemical Measurements

Peak CPK and creatine kinase MB levels were 4215 ± 1933 and 487 ± 255 IU/L, respectively. Medications at discharge were as follows: aspirin, 100%; statins 60%; angiotensin-converting enzyme inhibitors or angiotensin-receptor blockers, 60%; and β-blockers, 40%. After 3 month, in addition to no significant changes in left ventricular end-diastolic volume index, left ventricular end-systolic volume index, and left ventricular ejection fraction (LVEF), wall motion index in the infracted area was significantly improved at 3 months in comparison with the data at admission (Table 3). CMR data also showed that LVEF, EDVI, and ESVI at 3 months were not different with those at 1 month. These results were accompanied with the significant decrease in infarct mass index (26% enhanced myocardium) in comparison between 1 and 3 months. BNP levels increased from 28.5 ± 33.0 pg/mL at 24 hours to 138.9 ± 101.1 pg/mL at 1 week ($P < 0.05$) and 130.2 ± 81.3 pg/mL at 1 month ($P < 0.05$) but decreased to 93.9 ± 70.1 pg/mL at 3 months. However, the levels of IL-1β, IL-6, and TNF-α did not change among the 3 time points (at 24 hours, 1 week, and 1 month; data not shown). In-stent restenosis was found in 2 patients at 3-month follow-up.

DISCUSSION

This is the first clinical pilot study of AM administration in patients with first AMI, showing its therapeutic potential with beneficial hemodynamic effects.

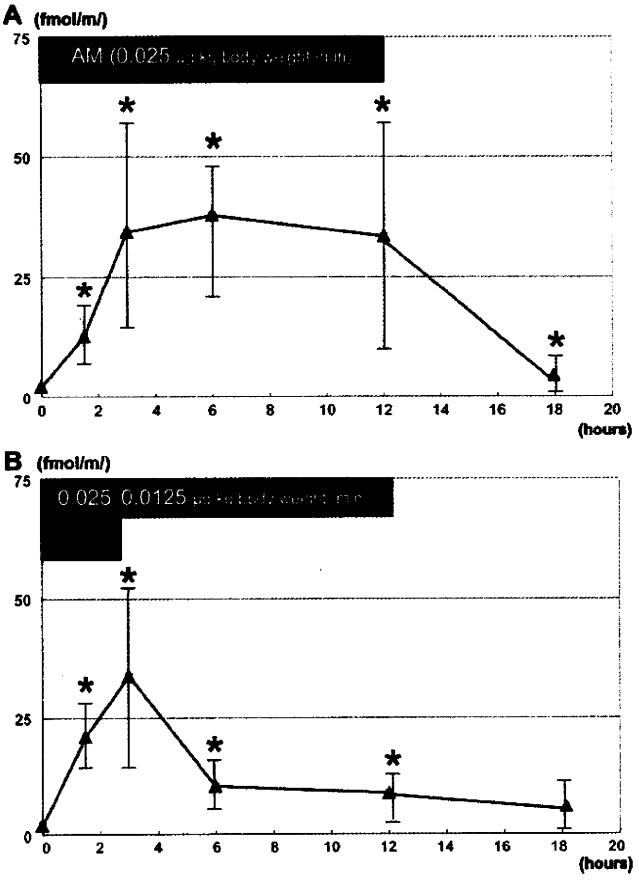

FIGURE 2. Changes in serum concentration of AM. A, Serum concentration of AM intravenously administered at the dose of 0.025 μg·(kg body weight)$^{-1}$·min^{-1} for 12 hours (n = 6). B, Serum concentration of AM intravenously administered at the dose of 0.025 μg·(kg body weight)$^{-1}$·min^{-1} for the first 3 hours and at the dose of 0.0125 μg·(kg body weight)$^{-1}$·min^{-1} for the following 9 hours (n = 4). Data shows mean ± SD. *$P < 0.05$ versus baseline.

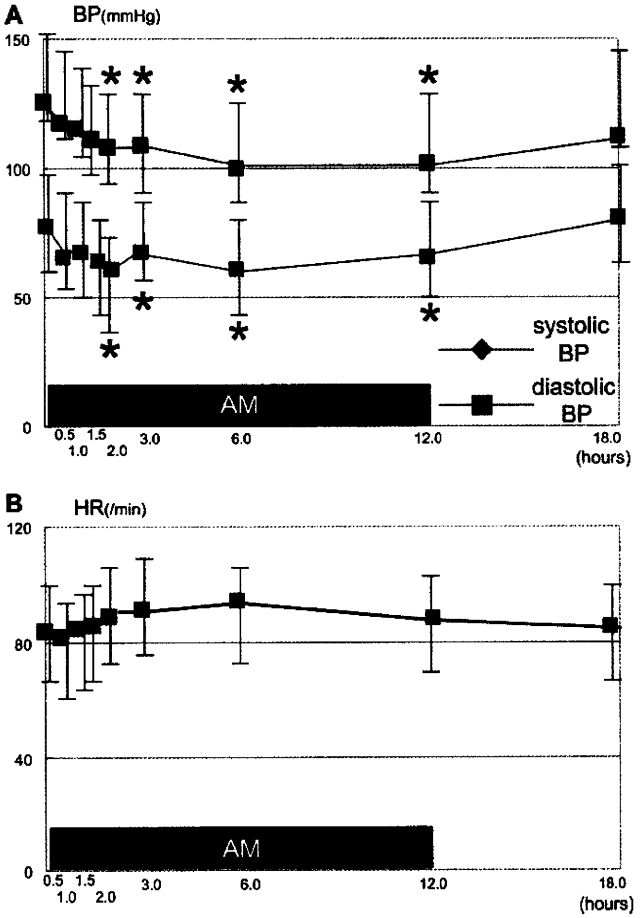

FIGURE 3. Changes in blood pressure and HR in response to AM administration in patients with AMI. A, systolic and diastolic blood pressure (BP). B, HR. Data shows mean ± SD. *$P < 0.05$ versus baseline.

Hemodynamic Effects of AM in Patients With AMI

Previous experimental and clinical studies revealed that AM could dilate pulmonary arterial resistance vessels and produces a positive inotropic action through cyclic adenosine monophosphate–independent mechanisms.[17–19] In fact, as shown in Figure 3, decrease in PCWP and mPA and increase in CI were induced during AM administration. These changes were consistent with previous results in patients with congestive heart failure and idiopathic pulmonary arterial hypertension.[20,21] Potent vasodilating effect of AM also decreased blood pressure to 20% from the baseline, without significant HR changes.

A Potential Cardioprotective Efficacy of AM for AMI

As shown in Figure 3, a reduction of blood pressure with minimizing HR change could decrease rate–pressure product and decreased oxygen consumption and would constitute a potential cardioprotective mechanism.[22] Previous experimental studies have also shown that AM has a variety of cardiovascular effects other than vasodilating effect.[8,9,23–26] In the previous experimental studies, AM administration attenuated myocardial/ischemia–reperfusion injury and exerted antiapoptic effects in cardiomyocytes by the Akt activation.[8,23] Nishida H et al[24] also demonstrated that AM administration reduced infarct size via phosphatidylinositol 3-kinase–mediated pathway. Moreover, AM could dilate human coronary arterioles and improve myocardial perfusion[25] and decrease ischemia–reperfusion arrhythmias.[26] Importantly, as shown in Table 2, high incidence of complete ST segment resolution and blush grade score more than 2 were observed after PCI. Reperfusion arrhythmias were unlikely to be induced.

It should be also noted that oxidant stress and several inflammatory cytokines play an important role in the pathological process of cardiac ischemia/reperfusion injury and remodeling.[27,28] In addition to anti-inflammatory effect,[29] AM exerts antioxidant effect with the suppression of oxygen-free radicals production.[9,10,13,23,26,30] In the present study,

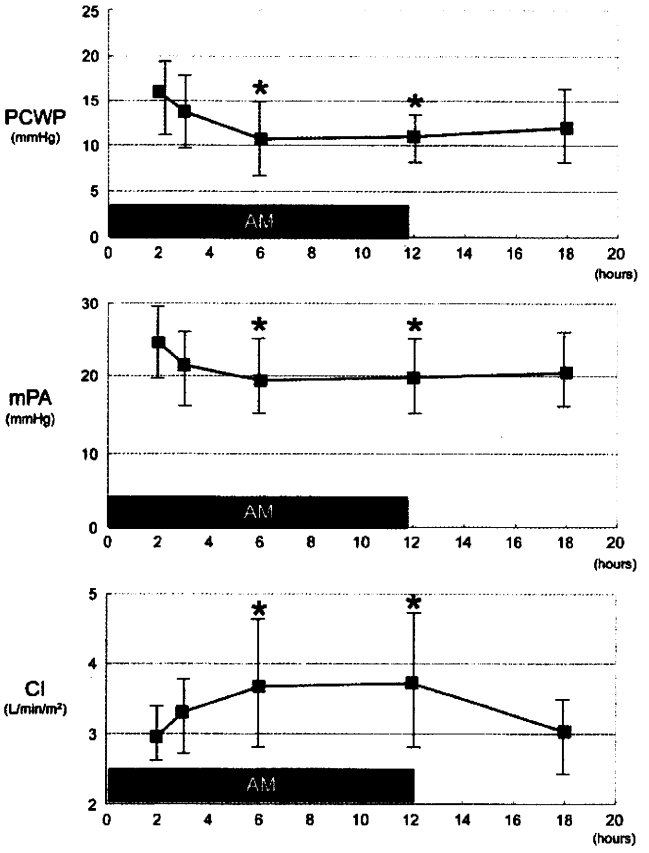

FIGURE 4. Changes in hemodynamic parameters in response to AM administration in patients with AMI. Data shows mean ± SD. *$P < 0.05$ versus baseline.

8-iso-PGF2α level in urine collecting for 24 hours after reperfusion was much lower than those previously reported (510 pmol/mmol creatinine in patients treated without AM).[26]

As shown in Table 3, CMR and LVG at 3 months showed the reduction of infarct size and the improvement of wall motion in the infracted area without significant changes in left ventricular end-diastolic volume index and LVEF. Importantly, CMR provides highly quantitative noninvasive measures of infarct size and seems to be more robust than the use of either nuclear medicine approaches or cardiac enzyme levels.[31] BNP, a biochemical marker for the left ventricular remodeling, was elevated to approximately 140 pg/mL at 7 days. However, BNP release was not sustained, rather decreased from approximately 130 pg/mL at 1 month to <100 pg/mL (−28% reduction on average) at 3 months. These findings were comparable with our previous study, in which patients with AMI showing decreasing BNP levels in the chronic phase despite high BNP levels at 7 days are unlikely to develop postinfarction ventricular remodeling.[32] However, because the absence of a control group makes it difficult to assess the efficacy of AM in patients with AMI, further studies are needed.

Adverse Effects of AM

The 2 of 10 patients experiencing adverse blood pressure reductions should be noted in the present study.

TABLE 3. LVG and CMR Data (n = 9)*

	Post-PCI	3 Mo	P
LVG data			
Volumetric analysis			
LVEF (%)	45.7 ± 5.4	46.1 ± 5.3	0.85
LVEDVI (mL/m²)	63.9 ± 18.1	67.2 ± 9.9	0.68
LVESVI (mL/m²)	35 ± 11.5	36.2 ± 6.2	0.77
Wall motion index score			
Noninfarct area	3.75 ± 0.23	3.37 ± 0.23	0.25
Infarct area	1.04 ± 0.22	1.76 ± 0.19	0.03
	1 Mo	**3 Mo**	**P**
CMR data			
LVEF (%)	43.0 ± 7.5	43.5 ± 7.0	0.29
LVEDVI (mL/m²)	73.7 ± 17.0	72.9 ± 8.7	0.41
LVESVI (mL/m²)	42.5 ± 12.9	41.3 ± 27.9	0.18
Left ventricular mass (g)	116.5 ± 21.5	116.8 ± 27.3	0.54
Enhanced myocardium mass (g)	25.9 ± 8.3	19.1 ± 6.3	0.09
Nonenhanced myocardium (g)	90.6 ± 18.4	97.7 ± 23.6	0.91
Percentage of enhanced myocardium (%)	22.3 ± 5.9	16.4 ± 4.2	0.03

*One patient who died of cardiac rupture in acute phase was not included.
LVEDVI, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; LVESVI, left ventricular end-systolic volume index.

Vasodilating effects of AM could deteriorate the hemodynamics of right ventricular infarction, probably due to decrease in right ventricular filling pressure,[33] although blood pressure recovered soon after AM discontinuation.

In the present study, cardiac rupture developed in one patient, in whom serum AM level was low at 3 hours after administration. AM decreases loading condition of left ventricle, which would rather predispose to prevent = cardiac rupture.[34] Furthermore, the previous experimental studies demonstrated that AM did not increase early mortality of AMI[30] and that AM could modulate wound healing process.[35] Therefore, it would be difficult to explain possible mechanism of AM to induce cardiac rupture in the clinical settings.

CONCLUSIONS

The present study demonstrated that intravenous administration of AM can be adjunctive to PCI, considering a variety of potential cardiovascular protective actions of AM (eg, attenuating ischemia–reperfusion injury/arrhythmias or suppressing oxygen-free radicals production). However, these data are preliminary and require confirmation in the future study.

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Common Variants in the ATP2B1 Gene Are Associated With Susceptibility to Hypertension: The Japanese Millennium Genome Project

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The Japanese Millennium Genome Project

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Abstract—Hypertension is one of the most common complex genetic disorders. We have described previously 38 single nucleotide polymorphisms (SNPs) with suggestive association with hypertension in Japanese individuals. In this study we extend our previous findings by analyzing a large sample of Japanese individuals (n=14 105) for the most associated SNPs. We also conducted replication analyses in Japanese of susceptibility loci for hypertension identified recently from genome-wide association studies of European ancestries. Association analysis revealed significant association of the *ATP2B1* rs2070759 polymorphism with hypertension ($P=5.3\times10^{-5}$; allelic odds ratio: 1.17 [95% CI: 1.09 to 1.26]). Additional SNPs in *ATP2B1* were subsequently genotyped, and the most significant association was with rs11105378 (odds ratio: 1.31 [95% CI: 1.21 to 1.42]; $P=4.1\times10^{-11}$). Association of rs11105378 with hypertension was cross-validated by replication analysis with the Global Blood Pressure Genetics consortium data set (odds ratio: 1.13 [95% CI: 1.05 to 1.21]; $P=5.9\times10^{-4}$). Mean adjusted systolic blood pressure was highly significantly associated with the same SNP in a meta-analysis with individuals of European descent ($P=1.4\times10^{-18}$). *ATP2B1* mRNA expression levels in umbilical artery smooth muscle cells were found to be significantly different among rs11105378 genotypes. Seven SNPs discovered in published genome-wide association studies were also genotyped in the Japanese population. In the combined analysis with replicated 3 genes, *FGF5* rs1458038, *CYP17A1*, rs1004467, and *CSK* rs1378942, odds ratio of the highest risk group was 2.27 (95% CI: 1.65 to 3.12; $P=4.6\times10^{-7}$) compared with the lower risk group. In summary, this study confirmed common genetic variation in *ATP2B1*, as well as *FGF5*, *CYP17A1*, and *CSK*, to be associated with blood pressure levels and risk of hypertension. **(*Hypertension*. 2010;56:973-980.)**

Key Words: hypertension ■ genetic variation ■ ATP2B1 ■ Millennium Genome Project ■ Global BPgen

Because of its large impact on a number of cardiovascular diseases, hypertension is a major contributor to global health burden. Because hypertension is one of the most prevalent complex genetic disorders, with a heritability of ≤60% based on the estimation by 24-hour blood pressure (BP) readings,[1] numerous studies, including recent genome-wide association studies (GWAS),[2–6] have attempted to identify genetic variation associated with human BP levels.

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Except for rare mendelian forms of hypertension,[7] the estimated effects of each genetic factor on BP levels have been found to be small in the general population (typically <1.0 mm Hg on systolic BP [SBP] and <0.5 mm Hg on diastolic BP [DBP] per risk allele). However, multiple risk alleles are known to have a cumulative impact on several complex traits, including BP and hypertension risk.[3] In addition, it is anticipated that identification of novel susceptibility genes would lead to further understanding of disease pathogenesis.

As a part of a series of nationally based cooperative projects, the Millennium Genome Project (Millennium GPJ), we conducted multiple candidate gene analyses to identify susceptible genes and polymorphisms for hypertension. In a previously reported study,[6] we focused on 307 genes, which were genes encoding components of signal transduction pathways potentially related to BP regulation, including receptors, soluble carrier proteins, binding proteins, channels, enzymes, and G proteins. That study identified 38 single nucleotide polymorphisms (SNPs) as suggestively associated with hypertension by analysis of 758 hypertensive patients and 726 normotensive controls.[6] To extend our previous study, we have now genotyped all 38 of the SNPs in a replication panel composed of 1929 hypertensives and 1993 normotensives and have taken forward validated SNPs with further genotyping in a large Japanese genetic epidemiological cohort sample (n=14 105). An in silico validation analysis of our most promising loci was performed using the Global Blood Pressure Genetics (Global BPgen) consortium data set, a large-scale GWAS of samples of European descent.[2] Furthermore, we also conducted a replication analysis of recent European GWAS-derived susceptible loci for hypertension from Global BPgen[2] and CHARGE (Cohorts for Heart and Aging Research in Genome Epidemiology) GWAS[3] in a Japanese large-scale general population sample (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).

Methods

Case and Control Subjects (Screening Panel)

Details of the screening panel subjects have been described previously.[6] Briefly, hypertensive patients and normotensive controls were recruited in the Asahikawa, Tokyo, Osaka, and Hiroshima regions of Japan according to the following criteria. Hypertensive subjects (n=758) had a previous diagnosis of hypertension at between 30 and 59 years of age and were either being treated with antihypertensive medication or had a SBP >160 mm Hg and/or DBP >100 mm Hg. They had a family history of hypertension in their parents and/or siblings and were not obese (body mass index [BMI] <25 kg/m^2). Normotensive controls (n=726) aged >45 years were recruited from the same regions. These individuals have never been treated with antihypertensive medications, and their SBP was <120 mm Hg and DBP <80 mm Hg. They had no family history of hypertension. All of the subjects were unrelated and were native Japanese.

Cohort-Based Population Samples

Seven independent study cohorts for cardiovascular diseases and related risk factors were combined to compose a large-scale Japanese genetic epidemiological population sample of 14 105. The Ohasama, Shigaraki, Takashima, Suita, and Nomura studies are general population-based genetic epidemiological studies. The study subjects were recruited via a medical checkup process for community residents. The 2 other cohorts, Yokohama and Matsuyama, are derived from employees of large manufacturing industries. The clinical parameters used in this study were obtained from personal health records during annual medical checkups. Further details of the study cohorts are described in the online Data Supplement.

Nested Case and Control Subjects Derived From the Cohort-Based Sample (Replication Panel)

Hypertensive cases and normotensive controls were chosen from the cohort-based population samples described above (n=11 569; the Suita study was excluded because of ethical issues). The selection criteria of the hypertensive and normotensive subjects were as follows: hypertensive subjects (n=1929) aged ≤64 years and either treatment with antihypertensive medication and/or SBP >160 mm Hg and/or DBP >90 mm Hg; normotensive subjects (n=1993) aged ≥40 years and having SBP <120 mm Hg and DBP <80 mm Hg; and no current use of antihypertensive medication and free from any history of cardiovascular disease.

Global BPgen (In Silico) Analyses

To investigate cross-validation of the most promising SNPs, we obtained results for 4 SNPs in the *ATP2B1* gene from the Global BPgen consortium, a study that is composed of 17 GWAS studies with 34 433 individuals of European descent. A detailed description of the study design and phenotype measurement for all of the cohorts has been reported previously.[2]

Validation of Published BP Polymorphisms in the Japanese Millennium Cohort

Thirteen loci have been identified recently and robustly validated for association with BP and hypertension in recent large-scale GWAS of European samples, by the Global BPgen consortium[2] and the CHARGE consortium.[3] From the associated SNPs reported at these 13 loci, we selected SNPs expected to have minor allele frequencies in Japanese samples >0.10, based on the HapMap database (JPT only, Public Release No. 27)[8]: *FGF5* rs1458038, *CYP17A1* rs1004467, *CSK* rs1378942, *PLCD3* rs12946454, *PLEKHA7* rs381815, *ULK4* rs9815354, and *CSK-ULK3* rs6495122. These 7 SNPs were genotyped in the Japanese population-based cohort sample to test whether the same associations exist in samples of Japanese ancestry.

Genotyping

Genomic DNA was extracted from peripheral blood. All of the SNPs were analyzed by TaqMan probe assays (Applied Biosystems Co, Ltd) using commercially available primers and probes purchased from the Assay-on-Demand system. The fluorescence level of PCR products was measured using an ABI PRISM 7900HT sequence detector.

Ethical Considerations

All of the study procedures were approved by the ethics committee of each university or research institute. Written informed consent was obtained from all of the participating subjects.

Ex Vivo Expression Analysis of ATP2B1 mRNA

Umbilical artery smooth muscle cells were isolated from umbilical cords obtained at delivery (n=34). Expression levels of ATP2B1 mRNA were analyzed by RT-PCR using a relative quantification method. Further details of the ex vivo expression analysis are described in the online Data Supplement.

Statistical Analysis

At each SNP, frequency differences in each genotype among hypertensive and normotensive subjects were assessed using a χ^2 test. Linkage disequilibrium (LD) coefficients were calculated using the Haploview software (Broad Institute).[9] Adjusted odds ratios for hypertension, as well as coefficients and SEs for SBP and DBP, were calculated using logistic and linear multiple regression analysis,

Table 1. Association of ATP2B1 SNPs With Hypertension in the Screening and Replication Panels

			Screening Panel						Replication Panel						
SNP	Genotype		Genotype Frequency			HWE	Call Rate	Odds (*P*)	Genotype Frequency			HWE	Call Rate	Odds (*P*)	Overall Odds (*P*)
rs1401982	AA/AG/GG	HT	318	328	92	0.603	96.3	1.28 (0.001)	825	833	247	0.108	98.7	1.25 (3.0×10^{-6})	1.26 (1.5×10^{-8})
		NT	249	324	118	0.474			699	961	305	0.397			
rs2681472	AA/AG/GG	HT	335	321	90	0.334	97.8	1.26 (0.003)	846	832	242	0.095	99.5	1.26 (1.0×10^{-6})	1.26 (8.7×10^{-9})
		NT	267	328	111	0.539			715	966	303	0.431			
rs2070759	GG/GT/TT	HT	216	379	151	0.515	97.6	1.16 (0.045)	582	896	399	0.118	97.2	1.18 (4.4×10^{-4})	1.17 (5.3×10^{-5})
		NT	186	341	175	0.454			507	956	474	0.579			
rs11105364	TT/TG/GG	HT	335	322	88	0.432	97.2	1.29 (0.001)	846	834	236	0.171	99.3	1.25 (2.4×10^{-6})	1.26 (4.1×10^{-9})
		NT	261	323	113	0.438			729	947	303	0.874			
rs11105378	CC/CT/TT	HT	359	301	76	0.276	97.3	1.37 (6.3×10^{-5})	868	821	217	0.280	98.8	1.28 (1.4×10^{-7})	1.31 (4.1×10^{-11})
		NT	280	320	108	0.295			746	922	300	0.586			

The screening panel is composed of 758 middle age–onset severe hypertensive patients and 726 middle-aged to elderly evidently normotensive controls (Table S4). The replication panel consists of 1929 hypertensive cases, and 1993 normotensive controls selected from 11 569 cohort sample were enrolled (Table S2). ORs and *P* values for allelic model are shown.

adjusting for sex, age, age^2, BMI, and cohort variables, using additive (1 degree of freedom) and genotypic (2 degrees of freedom) genetic models. 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).[10] The Global BPgen data and statistical methods have been described elsewhere.[2] Meta-analysis was performed assuming fixed effects and using inverse variance weights. An unweighted genetic risk score based on 4 SNPs (*ATP2B1* rs1105378, *FGF5* rs1458038, *CYP17A1* rs1004467, and *CSK* rs1378942) was calculated by adding the number of risk alleles showing higher BP values. Risk allele of each SNP was defined as follows: *ATP2B1*, C allele; *FGF5*, T allele; *CYP17A1*, A allele; and *CSK*, C allele. The *CSK-ULK3* SNP rs6495122 showing positive association with BP trait and hypertension was not included in the calculation of genetic risk score, because the strong LD with the *CSK* SNP rs1378942 ($D'=0.884$; $r^2=0.731$) is most parsimoniously explained by both SNPs tagging a single risk variant. Differences in mRNA expression levels among the *ATP2B1* rs1105378 genotype were assessed by ANOVA. The statistical analyses were performed using a commercially available statistical software package (JMP version 8, SAS Institute).

Results

Replication Genotyping

The clinical characteristics of the replication panel chosen from the cohort-based population samples (Table S1, available in the online Data Supplement) are shown in Table S2. Stringent case and control definitions, corresponding with the extreme upper ≈17% and lower ≈17% of the general population, were used to maximize power for fixed genotyping costs.[11] Thirty-six SNPs were successfully genotyped, and results for all of the SNPs are shown in Table S3. Significant association was observed for the *ATP2B1* rs2070759 polymorphism located in intron 8 ($P=4.4\times10^{-4}$; allele odds ratio [OR]: 1.18 [95% CI: 1.07 to 1.29]). Several other SNPs also showed marginally significant association; however, the *P* values did not reach statistical significance after application of Bonferroni correction for multiple comparisons (threshold: 0.05/36=0.0014; Table S3; we note that no other SNPs are significant if the less conservative Benjamini-Hochberg procedure is used to control the false discovery rate at 0.05). Although, the replication results in the less-strict nested case-control sample chosen from the same population sample have been reported in our previous article,[6] the association was recalculated to narrow down the SNPs to be applied to the following dense SNP analysis.

Dense SNP Analysis of the ATP2B1 Gene

To more precisely identify the SNP or SNPs increasing susceptibility for hypertension, we performed "de novo" genotyping of a dense SNP panel around marker rs2070759 in individuals from the original screening panel (Table S4).[6] Forty-one tag SNPs located in a 167-kb region around rs2070759 were selected using the HapMap database (Table S5).[8] Among the 27 SNPs polymorphic in our Japanese sample, the most significant association was observed with rs11105378; this yielded an allelic *P* value of 6.3×10^{-5} (OR: 1.37 [95% CI: 1.17 to 1.60]; Table 1 and Figure S2).

The most associated SNP and the 4 others from the dense SNP analyses were subsequently genotyped in the replication panel. Significant association of rs11105378 was confirmed in the replication panel with an allelic *P* value of 1.4×10^{-7} (OR: 1.28 [95% CI: 1.17 to 1.41]; Table 1). Meta-analysis of both study panels indicated significant association ($P=4.1\times10^{-11}$; OR: 1.31 [95% CI: 1.21 to 1.42]) and confirmed that the strongest association is seen for rs11105378. The D' and r^2 measures of LD between rs2070759 and rs11105378 were 0.92 and 0.48, respectively. Other SNPs, rs1401982 ($D'=0.99$; $r^2=0.64$), rs2681472 ($D'=0.99$; $r^2=0.61$), rs11105364 ($D'=0.97$; $r^2=0.59$), located within the same LD block, were also significantly associated with hypertension (Table 1). The strong LD between associated SNPs suggests a single true association signal in this region.

We examined for possible association of SNPs in the *ATP2B4* gene, a well-investigated isoform of the *ATP2B1* gene, with hypertension in the screening panel. We observed no significant correlation with the 17 SNPs analyzed, which were selected using the HapMap database (Table S6).

Population-Based Meta-Analyses of ATP2B1 SNPs

The complete Japanese population-based sample was subsequently genotyped for the 4 most significant SNPs in