increased in masked hypertension compared with white-coat hypertension. Body mass index, duration of hypertension, the prevalence of diabetes mellitus and hyperlipidemia, and the rate of current smokers did not differ among the four groups. In addition, there were no intergroup differences in metabolic parameters and renal function, except that triglyceride level was somewhat increased in masked hypertension.

As for antihypertensive treatment, the period of medication was significantly shorter in masked hypertension than in white-coat hypertension, probably reflecting that the mean age of the group with masked hypertension was lowest. The percentage of the use of Ca channel blockers was significantly higher in white-coat hypertension, masked hypertension, and sustained hypertension than in controlled hypertension. The percentage of treatment with β-blockers or diuretics and that of combination treatment were lower in sustained hypertension than in white-coat hypertension or masked hypertension. The use of angiotensin II receptor antagonists or angiotensin-converting enzyme inhibitors and total number of classes of antihypertensive drugs did not differ among the four groups.

As shown in Table 2, clear differences in office and ambulatory BP levels were observed among the groups with controlled, white-coat, masked, and sustained hypertension. The standard deviations of ambulatory daytime and night-time BP values were significantly increased in masked hypertension compared with controlled hypertension or white-coat hypertension. The degree of nocturnal dipping in systolic BP was significantly larger in masked hypertension than in controlled and white-coat hypertension.

The LV and carotid arterial structural changes and U-Alb levels in the four groups are shown in Fig. 1. The LV mass index (g/m^2) was significantly increased in masked hypertension (134 ± 29) than in controlled hypertension (115 ± 34) and white-coat hypertension (119 ± 32). Its level in sustained hypertension (126 ± 32) was significantly higher only compared with that in controlled hypertension. There was no difference in conventional IMT among the four groups (data not shown). However, maximal IMT (mm), which more sensitively reflects the severity of carotid atherosclerosis than conventional IMT,[16] was significantly greater in masked hypertension (1.93 ± 1.07) than in controlled hypertension (1.61 ± 0.67) and white-coat hypertension (1.60 ± 0.82). The level in sustained hypertension (1.69 ± 0.92) was not significantly higher compared with those in controlled and white-coat hypertension. The patients with masked hypertension tended to have more increased LV mass index and maximal IMT than those with sustained hypertension ($P < .10$, respectively). The U-Alb levels ($\log_{10}$ mg/g Cr) were significantly higher in masked (1.43 ± 0.62) and sustained hypertension (1.42 ± 0.55) than in controlled (1.12 ± 0.43) and white-coat hypertension (1.22 ± 0.47), and the

Table 2. Office and ambulatory blood pressure (BP), heart rate, and BP variability in study patients

Characteristic	Controlled hypertension (n = 51)	White-coat hypertension (n = 65)	Masked hypertension (n = 74)	Sustained hypertension (n = 142)
Office BP (mm Hg)				
Systolic	130 ± 6	152 ± 16*	130 ± 6†	155 ± 15*‡
Diastolic	75 ± 8	86 ± 12*	77 ± 7†	88 ± 11*‡
Ambulatory BP (mm Hg)				
24-h Systolic	125 ± 7	128 ± 9	137 ± 8*†	144 ± 13*†‡
24-h Diastolic	72 ± 7	74 ± 7	82 ± 8*†	83 ± 10*†
Daytime systolic	127 ± 6	129 ± 6	142 ± 7*†	147 ± 14*†‡
Daytime diastolic	73 ± 6	76 ± 7	85 ± 9*†	86 ± 11*†
Night time systolic	120 ± 11	121 ± 12	129 ± 13*†	137 ± 16*†‡
Night time diastolic	68 ± 8	70 ± 9	77 ± 9*†	78 ± 11*†
Heart rate (beats/min)				
Office	67 ± 10	69 ± 9	68 ± 9	70 ± 9*
24-h	64 ± 9	63 ± 11	68 ± 9*†	68 ± 10*†
Daytime	67 ± 10	66 ± 10	70 ± 10†	70 ± 11*†
Night time	58 ± 8	59 ± 9	62 ± 9*†	62 ± 9*†
SD of ambulatory BP (mm Hg)				
Daytime systolic	14.2 ± 3.5	13.7 ± 2.8	15.0 ± 3.8†	14.7 ± 4.6
Daytime diastolic	9.9 ± 3.1	9.5 ± 2.7	10.8 ± 3.4*†	10.1 ± 2.9
Night time systolic	11.6 ± 3.4	10.8 ± 3.7	12.0 ± 3.9†	11.4 ± 3.3
Night time diastolic	8.6 ± 2.3	7.9 ± 2.8	9.0 ± 2.5†	8.5 ± 2.4
Nocturnal BP dipping (%)				
Systolic	5.6 ± 8.1	5.6 ± 8.3	8.7 ± 7.7*†	6.7 ± 8.6
Diastolic	7.0 ± 8.6	7.6 ± 8.4	9.9 ± 7.7	8.4 ± 8.7

Values are mean ± SD.
* $P < .05$ *v* controlled hypertension; † $P < .05$ *v* white-coat hypertension; ‡ $P < .05$ *v* masked hypertension.

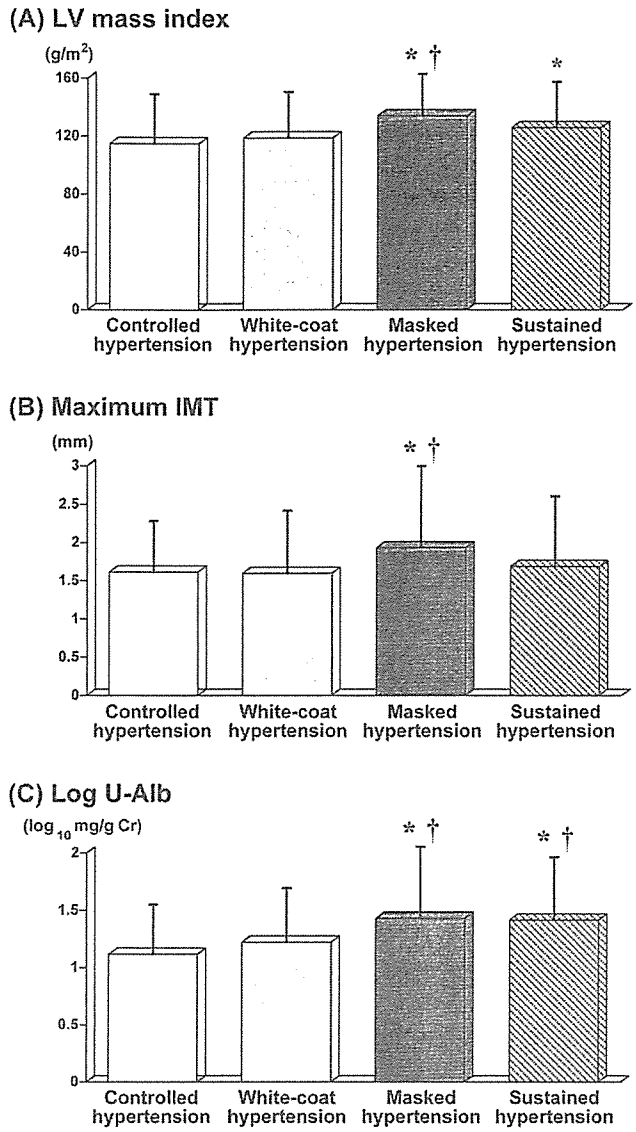

FIG. 1. Left ventricular (LV) mass index (**A**), maximal intima-media thickness (IMT) (**B**), and urinary albumin (U-Alb) (log scale, C) in the four study groups, divided by office and daytime ambulatory blood pressure levels. Values are given as mean ± SD. *$P < .05$ *v* controlled hypertension; †$P < .05$ *v* white-coat hypertension.

levels in masked hypertension and sustained hypertension were almost the same.

To identify independent predictors for target organ changes, we investigated possible determination factors using a stepwise multiple regression analysis in all subjects. Although daytime systolic BP was a strong predictor for LV mass index, maximal IMT, and U-Alb levels, the presence of masked hypertension was found to be one of the independent determinants of these end-organ changes (Table 3).

Discussion

There have been a few studies reporting the possible association between masked hypertension and cardiac and carotid arterial structural changes in the general population. In a cross-sectional study, Liu et al[18] found that LV mass and carotid wall thickness in patients with masked hypertension were significantly greater than those in true normotensive subjects and similar to those in patients with sustained hypertension. The data from the Pressione Arteriose Monitorate E Loro Associazioni (PAMELA) Study also showed that LV mass index was increased in untreated subjects with isolated ambulatory hypertension and sustained hypertension than in those with true normotension.[19] The present findings were broadly consistent with these previous observations. Therefore, our study suggests that a higher level of ambulatory BP largely affects target organ damage in treated hypertensive patients as well as in untreated subjects. In the present study, however, the average levels of 24-h, daytime, and night-time ambulatory BP in the masked hypertension group were somewhat lower than those in the sustained hypertension group. In addition, the presence of masked hypertension was a significant predictor for end-organ changes, independent of average daytime BP levels. Thus factors other than a higher ambulatory BP could contribute to target organ damage in masked hypertension. A shorter period of antihypertensive medication might partially explain the advanced target organ changes in patients with masked hypertension.

In the present study, 22% of subjects were identified as having masked hypertension, which was associated with a higher proportion of men and younger age. These characteristics observed in our study are in agreement with those of masked hypertension described in other studies.[20,21] Increased physical and mental activities in younger men are likely to induce the augmentation of daytime BP variability, which might promote cardiac, carotid arterial, and renal damage in masked hypertension, because several studies have shown that short-term BP variability, apart from average ambulatory BP values, is associated with target organ damage in hypertensive patients.[22–25]

Two recent large-scale prospective studies revealed that a high ambulatory or home BP is a powerful predictor for cardiovascular morbidity in patients with treated hypertension even when their office BP is well controlled. One study by Clement et al[10] showed that the relative risk of cardiovascular events associated with a high 24-h ambulatory systolic BP (≥135 mm Hg) as compared with a low 24-h systolic BP (<135 mm Hg) was 3.19 (unadjusted) or 2.80 (after adjustment) among patients with an office systolic BP of <140 mm Hg. In another cohort study by Bobrie et al,[11] the incidences of cardiovascular events in patients with controlled hypertension (office BP <140/90 mm Hg and home BP <135/85 mm Hg), elevated BP in the office but not at home (ie, white-coat hypertension), elevated BP at home but not in the office (ie, masked hypertension), and uncontrolled hypertension (ie, sustained hypertension) were 11.1, 12.1, 30.6, and 25.6 cases per 1000 patient-years, respectively. The hazard ratio of cardiovascular events in the group with masked hyperten-

Table 3. Independent predictors for target organ damage by multivariate regression analysis

Characteristic	β-Coefficient	F value	P value
LV mass index			
Daytime systolic BP	0.270	25.64	<.0001
Sex (male)	0.190	13.53	.0002
Presence of masked hypertension	0.136	6.56	.0101
Daytime heart rate	−0.135	6.25	.0112
Duration of hypertension	0.119	5.69	.0164
Body mass index	0.110	5.22	.0268
SD of daytime systolic BP	0.102	4.76	.0476
	$R^2 = 0.240$, $F = 14.37$, $P < .0001$		
Maximum IMT			
Age	0.342	37.47	<.0001
Daytime systolic BP	0.233	14.91	.0001
Daytime diastolic BP	−0.252	12.51	.0006
Sex (male)	0.184	11.38	.0008
Presence of masked hypertension	0.157	10.04	.0025
Current smoking	0.120	6.09	.0225
	$R^2 = 0.275$, $F = 12.42$, $P < .0001$		
Log U-Alb			
Daytime systolic BP	0.237	18.38	<.0001
Use of Ca channel blocker	0.166	8.84	.0035
Creatinine clearance	−0.168	8.82	.0030
Period of antihypertensive medication	0.159	7.16	.0067
Presence of diabetes mellitus	0.126	5.08	.0232
Presence of masked hypertension	0.114	4.02	.0421
	$R^2 = 0.205$, $F = 11.77$, $P < .0001$		

BP = blood pressure; Ca = calcium; IMT = intima-media thickness; LV = left ventricular; SD = standard deviation; U-Alb = urinary albumin.
The stepwise regression model included age, sex, body mass index, duration of hypertension, diabetes mellitus, hyperlipidemia, current smoking, habitual drinking, creatinine clearance, period of antihypertensive medication, use of each class of antihypertensive drug (Ca channel blocker, angiotensin II receptor blocker, angiotensin converting enzyme inhibitor, β-blocker, or diuretic), daytime systolic BP, daytime diastolic BP, daytime heart rate, SD of daytime systolic BP, SD of daytime diastolic BP, white-coat hypertension, masked hypertension, and sustained hypertension, as possible independent variables.

sion was shown to be greatest among the four subgroups by an analysis with the multivariable Cox model. Interestingly, our present findings were consistent with these observations examining the prognostic significance of masked hypertension in treated hypertensive subjects. Therefore, the progression of end-organ damage induced by masked hypertension may lead to the high incidence of cardiovascular events in such patients.

There were some limitations in our study. The sample size of our subjects might be relatively small to evaluate properly the differences in target organ damage among the four groups of patients. In addition, the present findings were derived from cross-sectional data on the basis of one-time examination of ambulatory BP monitoring, cardiac and carotid ultrasonography, and urinalysis. Thus a prospective study using larger population of hypertensive subjects will be required to confirm the influence of masked hypertension on target organ damage.

All patients in the present study had received antihypertensive medication. As another limitation of this study, we must therefore consider the possibility that different classes of antihypertensive drugs may have differently affected the development of target organ damage, partly independently of their BP-lowering effects. Renin-angiotensin system inhibitors, above all, have been known to have BP fall–independent protective effects on hypertensive target organ, although the percentage of patients treated with angiotensin II receptor antagonists or angiotensin converting enzyme inhibitors did not differ among the four study groups. Our multivariate analysis showed that the association of masked hypertension with target organ damage was independent of the use of any class of antihypertensive agent. However, approximately 60% of the present subjects were under combination drug treatments. In those cases, the possible specific effect of one or another class of antihypertensive drug could hardly be account for.

In conclusion, the present study shows that masked hypertension is associated with increased LV mass, carotid IMT, and albuminuria in patients with treated essential hypertension, and that the impact of masked hypertension on such end-organ changes is greater than that of controlled hypertension or white-coat hypertension and comparable to that of sustained hypertension. Masked hypertension as well as uncontrolled hypertension is a significant risk for target organ damage in treated hypertensive patients and ambulatory BP monitoring seems to be necessary to unmask this latent risk that is not detectable by routine BP measuring in the office.

Acknowledgments

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Adiponectin and Renal Function, and Implication as a Risk of Cardiovascular Disease

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The relation among adiponectin, renal function, and incident cardiovascular disease (CVD) in patients with different degrees of renal dysfunction was investigated. In total, 150 subjects were included in this study and followed prospectively for a mean of 32 months. At baseline, median adiponectin levels for chronic kidney disease (CKD) stages 1, 2, 3, 4 and 5, as estimated by creatinine clearance (≥90, 60 to 90, 30 to 60, <30 ml/min), were 3.06, 4.04, 6.43, and 11.9 μg/ml, respectively (p for trend <0.01), and a significant association between adiponectin and CKD stages was also confirmed in multivariate regression analysis (F = 6.2, p <0.001). During follow-up, 31 subjects developed CVD, including myocardial infarction, angina pectoris, stroke, and transient ischemic attack. Gender-specific median values of adiponectin were used to separate the higher group from the lower group, and the Kaplan-Meier curve showed a significantly lower event-free survival rate in the lower adiponectin group (<4.39 μg/ml in men, <6.84 μg/ml in women, chi-square 4.88, p <0.03). The risk factor-adjusted Cox regression showed that an increase in adiponectin per 1 μg/ml was associated with a decrease in the risk of CVD to 0.86 (95% confidence interval 0.75 to 0.96, p = 0.004). In the subgroup with previous ischemic heart disease (IHD; n = 65), a significantly lower event-free survival rate of IHD was also observed in the lower adiponectin group (<4.45 μg/ml in men, <4.49 μg/ml in women, chi-square 3.96, p <0.05). The relative distribution of adiponectin isoforms was examined in patients with severe CKD, and the percentage of the high-molecular-weight form in patients with IHD during follow-up (n = 3) was significantly smaller than that in those without IHD (n = 4, p <0.02). In conclusion, renal function is a significant regulator of adiponectin when categorized by CKD stage, whereas hypoadiponectinemia is a predictor of CVD, including recurrent IHD. (Am J Cardiol 2006;98:1603–1608)

Adiponectin is a key molecule in the metabolic syndrome[1–3] and is importantly involved in cardiovascular disease (CVD).[2,4] Recent data have suggest that hypoadiponectinemia is a novel putative CVD risk factor even in patients with mild to moderate renal failure,[5] although it has not been fully elucidated as to whether adiponectin concentration could be used as a predictive marker of CVD, separate from its increase by renal dysfunction.[6,7] Further, the clinical importance of adiponectin as a predictor of recurrent ischemic heart disease (IHD) has not been addressed. Thus, to examine whether renal function affects adiponectin concentration biologically, we carried out cross-sectional and longitudinal studies and evaluated its predictive power for CVD. Further, because different adiponectin isoforms have been reported to have certain clinical implications,[8] we examined the relative abundance of adiponectin isoforms in patients with severe chronic kidney disease (CKD).

Methods

Subjects: In total, 150 subjects were selected from among patients who were admitted and underwent medical investigation at the National Cardiovascular Center in Osaka, Japan. IHD was defined as ≥75% organic stenosis of ≥1 major coronary artery confirmed by coronary angiography or a history of myocardial infarction. All subjects with IHD in this study had undergone percutaneous transluminal coronary angioplasty before the initial assessment. Diabetes mellitus was defined according to criteria of the American Diabetes Association.[9] Hypertension was defined as a systolic blood pressure ≥140 mm Hg and/or a diastolic blood pressure ≥90 mm Hg on repeated measurements or use of antihypertensive treatment. Smoking was defined as current smoking or a history of habitual smoking. Subjects with acute coronary syndrome, cardiogenic shock, hemodialysis treatment, nephrotic syndrome, overt congestive heart failure, valvular heart disease, or atrial fibrillation were excluded. Further, no subjects receiving erythropoietin or steroid therapy were included in this study. All procedures in the present study were carried out in accordance with institutional and national ethical guidelines for human stud-

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ies. All subjects enrolled in this study were Japanese and gave informed consent to participate in the study.

Laboratory measurements: After subjects fasted overnight, blood pressure was measured by well-trained physicians with a mercury column sphygmomanometer, and venous blood was drawn from all subjects. Height and body weight were measured, and body mass index was calculated. Plasma samples for subsequent assay were stored at −80°C. Plasma concentration of adiponectin was determined by a sandwich enzyme-linked immunosorbent assay system (Adiponectin Enzyme-Linked Immunosorbent Assay Kit, Otsuka Pharmaceutical Co. Ltd., Tokyo, Japan) as previously reported.[1] Urine samples were collected for 3 days and averaged to evaluate creatinine clearance. The parameters hemoglobin, hematocrit, total cholesterol, triglycerides, and high-density lipoprotein cholesterol were also determined.

Follow-up study: After the initial assessment, patients were monitored for 31.9 ± 1.5 months. During follow-up, CVD events were accurately recorded. CVD events of interest in this study were myocardial infarction and angina pectoris confirmed by electrocardiographic changes; coronary angiographic and/or myocardial scintigraphic findings; stroke and transient cerebral ischemia confirmed by clinical symptoms; and computed tomographic, magnetic resonance angiographic, and/or cerebrovascular angiographic findings. IHD events included angina pectoris and myocardial infarction. In addition, restenosis of a lesion that had previously been subjected to percutaneous transluminal angioplasty at the initial assessment was not included as a CVD or IHD event for this analysis. Cause of death was classified as CVD, if there was sudden death from CVD, by an independent review panel of physicians who were unaware of echocardiographic and clinical findings. Events that were more equivocal, such as unrecognized myocardial infarction, angina pectoris, and transient cerebral ischemia, were not included as CVD for this analysis. For patients who developed multiple nonfatal episodes of CVD, the analysis included only the first event.

Because kidney transplantation affects adiponectin concentration,[10] patients receiving kidney transplants throughout follow-up were excluded from this study.

Analysis of oligometric state of adiponectin in plasma: Seven men (65.7 ± 3.2 years of age; body mass index 22.0 ± 1.5 kg/m^2) who were hospitalized in the National Cardiovascular Center were enrolled in this study. Methods of blood sampling and exclusion criteria of this study were identical to those previously described. Further, in this study, no subjects with CVD at the initial assessment were included and did not receive renal replacement therapy throughout follow-up. The relative isoform distribution of adiponectin was determined as previously reported.[8]

Statistical analysis: Data are expressed as mean ± SE. Levels of adiponectin were log-transformed for linear regression models, and relations between adiponectin and various parameters were assessed using univariate linear regression analysis and the Pearson correlation coefficient. Levels of adiponectin were assessed according to baseline creatinine clearance. Patients were categorized into 4 groups according to category of CKD as defined by Kidney Disease Outcomes Quality Initiative (K/DOQI) clinical practice guidelines for CKD as approximated by creatinine clearance (≥90, 60 to 90, 30 to 60, <30 ml/min),[11] i.e., groups 1 to 5, and then the significance of differences between groups was evaluated using 1-way of analysis of variance with the Dunnett multiple comparison post test. Multiple regression models were used to assess the relation between adiponectin levels and CKD groups after adjustment for age, gender, smoking habits, body mass index, hypertension, diabetes, total cholesterol, triglycerides, high-density lipoprotein cholesterol, and hemoglobin.

All subjects were categorized into 2 groups according to median baseline adiponectin level by each gender. Event-

Table 1
Clinical characteristics of total subjects (n = 150)

Variable	
Age (yrs)	67.7 ± 0.8
Men/women	102/48
Body mass index (kg/m^2)	23.5 ± 0.3
Smoker	72.7%
Previous IHD	43.3%
Diabetes mellitus	43.3%
Hypertension	87.3%
Adiponectin (μg/ml)*	4.8 (3.0, 9.8)
Hemoglobin (g/L)	128 ± 2
Hematocrit	0.39 ± 0.01
Systolic blood pressure (mm Hg)	139 ± 2
Diastolic blood pressure (mm Hg)	74 ± 1
Total cholesterol (mg/dl, mmol/L)	191 ± 3, 4.93 ± 0.07
Triglycerides (mg/dl, mmol/L)	112 ± 4, 1.27 ± 0.05
HDL cholesterol (mg/dl, mmol/L)	44.9 ± 1.2, 1.16 ± 0.03
Creatinine clearance (ml/min)	66.3 ± 3.5
CKD stage	
1	24.7%
2	32.0%
3	23.3%
4–5	20.0%

Values are means ± SEs.
* Values are medians (first and third quartiles).
HDL = high-density lipoprotein.

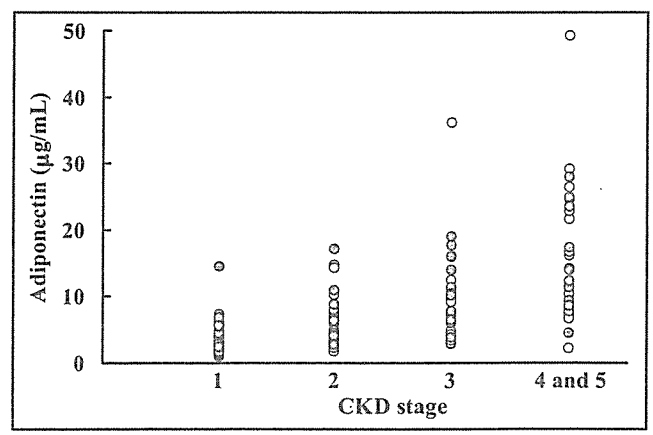

Figure 1. Baseline levels of adiponectin according to CKD stages in men (n = 102) *(gray circles)* and women (n = 48) *(white circles)*.

Table 2
Chronic kidney disease categories as a predictor of log-transformed adiponectin levels in the study

CKD Categories	Crude Model		Adjusted Model‡	
	Adiponectin* (μg/ml)	p Value	Mean Adiponectin† (μg/ml)	p Value
1	3.1 (2.2, 4.7)		5.4 ± 1.1	
2	4.0 (2.8, 6.8)	<0.05	5.7 ± 1.0	NS
3	6.4 (4.4, 11.1)	<0.01	8.9 ± 1.0	<0.01
4–5	11.9 (8.2, 23.0)	<0.01	12.1 ± 1.6	<0.01

* Values are presented as median (first and third quartiles).
† Values are presented as mean ± SE.
‡ Adjusted by age, gender, body mass index, smoker, diabetes, hypertension, previous IHD, triglycerides, high-density lipoprotein cholesterol, and hemoglobin.

Table 3
Clinical characteristics of subjects in follow-up study

Variable	Group 1* (n = 76)	Group 2† (n = 74)
Age (yrs)	69.4 ± 1.1	65.9 ± 1.1§
Men/women	51/25	51/23
Body mass index (kg/m^2)	22.1 ± 0.5	24.9 ± 0.5‖
Smoker	71.1%	74.3%
Previous IHD	43.4%	43.2%
Diabetes mellitus	42.1%	44.6%
Hypertension	86.8%	87.8%
Adiponectin (μg/ml)‡	6.9 (4.7, 9.5)	2.4 (1.8, 3.0)‖
Hemoglobin (g/L)	117 ± 2	139 ± 2‖
Hematocrit	0.36 ± 0.01	0.42 ± 0.01‖
Systolic blood pressure (mm Hg)	135 ± 2	142 ± 2§
Diastolic blood pressure (mm Hg)	74 ± 1	74 ± 1
Total cholesterol (mg/dl, mmol/L)	191 ± 4, 4.95 ± 0.10	190 ± 4, 4.91 ± 0.10
Triglycerides (mg/dl, mmol/L)	105 ± 5, 1.18 ± 0.06	121 ± 5§, 1.37 ± 0.06§
HDL cholesterol (mg/dl, mmol/L)	47.2 ± 1.2, 1.22 ± 0.04	42.5 ± 1.2§, 1.10 ± 0.04§
Creatinine clearance (mL/min)	42.2 ± 4.6	89.4 ± 4.7‖
No. of CVD events	12	19

Values are presented as mean ± SE.
* Adiponectin ≥4.39 μg/ml in men, ≥6.84 μg/ml in women.
† Adiponectin <4.39 μg/ml in men, <6.84 μg/ml in women.
‡ Values are presented as median (first and third quartiles).
§ p <0.05; ‖ p <0.01 versus group 1.
Abbreviation as in Table 1.

free survival analysis was performed with the Kaplan-Meier method to plot the cumulative incidence of CVD, and groups were compared by the Mantel log-rank test. Cox proportional hazard analysis was used to examine the association between variables and the cumulative incidence of CVD. Hazard ratios and their 95% confidence intervals were calculated using estimated regression coefficients and their SEs in Cox regression analysis.

In adiponectin isoform, statistical significance was tested using unpaired *t* tests. A p value <0.05 was considered statistically significant. All calculations were performed using JMP 4.0 (SAS Institute, Cary, North Carolina).

Results

Relation between adiponectin and renal dysfunction: Clinical and biochemical characteristics of study subjects are presented in Table 1. Adiponectin was significantly correlated with age (r = 0.31, p <0.01), body mass index (r = −0.33, p <0.01), hemoglobin (r = −0.63, p <0.01), hematocrit (r = −0.60, p <0.01), systolic blood pressure (r = −0.30, p <0.01), triglycerides (r = −0.35, p <0.01), high-density lipoprotein cholesterol (r = 0.29, p <0.01), and creatinine clearance (r = −0.65, p <0.01), and was decreased in men (7.1 ± 0.7 vs 9.1 ± 1.0 μg/ml, p <0.01) and smokers (7.4 ± 0.7 vs 8.6 ± 1.1 μg/ml, p <0.05). Figure 1 shows plots of patient adiponectin levels according to CKD categories at enrollment. Table 2 lists cross-sectional data regarding baseline CKD categories as predictors of log-transformed adiponectin levels. In crude and adjusted models, increasing categories of CKD were significant predictors of adiponectin levels (p for trend <0.01 for the 2 comparisons).

Plasma adiponectin concentration and CVD events: During follow-up, 31 patients (5 women) developed CVD. There were 2 with myocardial infarction, 13 with angina pectoris, 9 with cerebral infarction, and 7 with transient cerebral ischemia. Adiponectin was significantly lower in subjects

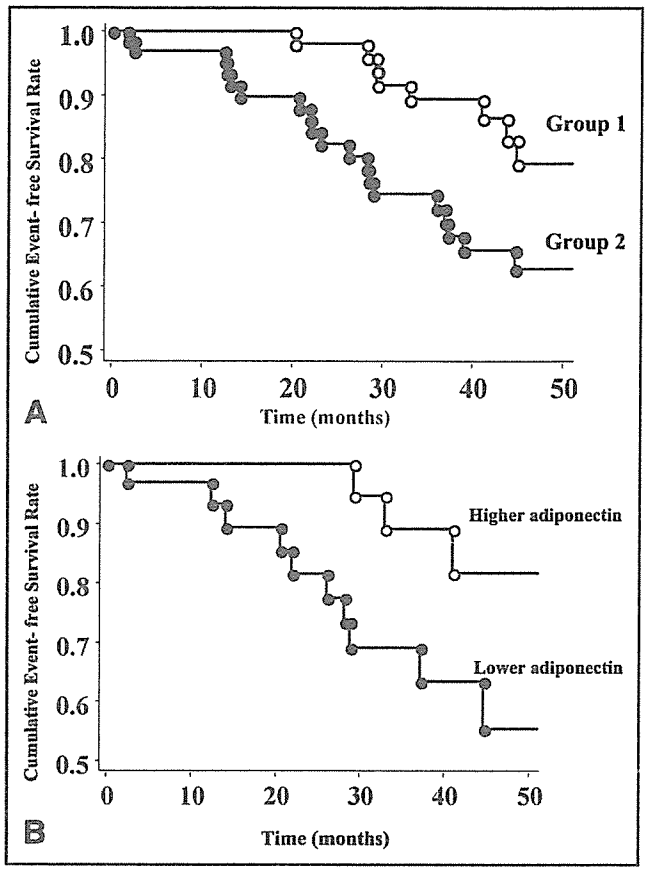

Figure 2. Kaplan-Meier survival curves for *(A)* CVD events in all subjects (n = 150, log-rank chi-square 4.88, p <0.03) and *(B)* IHD events in the subgroup with previous IHD (n = 65, log-rank chi-square 3.96, p <0.05). Subjects were stratified into 2 groups according to median adiponectin concentration by each gender.

who developed CVD events during follow-up (5.0 ± 1.3 vs 8.4 ± 0.7 μg/ml, p <0.02). Because of the gender-specific difference in adiponectin levels,[12] different median values for men and women were used to separate the higher group from the lower group of adiponectin (4.39 μg/ml in men, 6.84 μg/ml in women). Their clinical characteristics are listed in Table 3. Age, adiponectin, body mass index, hemoglobin, hematocrit, triglycerides, high-density lipoprotein cholesterol, and creatinine clearance were significantly different between the higher adiponectin group (group 1) and lower adiponectin group (group 2). Figure 2 shows the life-table analysis of CVD events throughout the follow-up period using the Kaplan-Meier method. These curves illustrate significantly poorer event-free survival of group 2. Results of Cox proportional hazard models are presented in Table 4, and the predictive value of adiponectin for CVD events in crude, CKD stage-adjusted, and risk factor-adjusted models are presented. Because previous IHD and smoking habit were also associated with a higher risk of CVD events, we selected these parameters as risk factors. Even after adjusting for CKD stages, previous IHD, and smoking habit, decreased adiponectin levels were a significant predictor of CVD in all models. The risk factor-adjusted Cox regression showed that an increase in adiponectin per 1 μg/ml was associated with a decrease in the risk of CVD to 0.86.

When the analysis was restricted to subjects with previous IHD (n = 65), 15 recurrent IHD events occurred during follow-up. Even in these subjects, adiponectin showed a tendency to be decreased in subjects who developed recurrent IHD events during follow-up (4.3 ± 1.9 vs 7.8 ± 1.1 μg/ml, p = 0.06). Different median values of adiponectin for men and women were also used to separate the higher group from the lower group (4.45 μg/ml in men, 4.49 μg/ml in women), and life-table analysis of recurrent IHD events throughout follow-up was performed using the Kaplan-Meier method (Figure 2). These curves showed significantly lower event-free survival of the lower adiponectin group. In addition, in Cox regression analysis, lower adiponectin was associated with a 1.85-fold higher risk of recurrent IHD events (hazard ratio 1.85, 95% confidence interval 1.02 to 3.92, p <0.05).

Relative plasma adiponectin isoform levels in patients with severe CKD: The ratio of the 3 major isoforms of adiponectin was analyzed in 7 men with severe CKD (creatinine clearance 8.9 ± 2.1 ml/min), and 3 IHD events occurred during follow-up. Adiponectin concentration was significantly lower in the subjects who had IHD events during follow-up (9.1 ± 0.1 vs 19.2 ± 2.9 μg/ml, p <0.04). Averaged elution profiles of adiponectin forms in plasma (Figure 3) and the percentage of each form of adiponectin in total adiponectin (Figure 3) are shown. The percentage of the high-molecular-weight form in total adiponectin was significantly lower in subjects who had IHD than in those who did not (−16.9%, p <0.02; Figure 3), whereas those of the hexamer and trimer in total adiponectin were not significantly different between the 2 groups. In addition, in the subjects without IHD throughout the follow-up period, the percentage of high molecular weight was significantly higher than that of the hexamer and trimer (vs hexamer +18.9%, p <0.05, vs trimer +39.6%, p <0.01; Figure 3).

Discussion

The present study demonstrated that renal function categorized by CKD stage was independently associated with adiponectin concentration. However, low adiponectin was a predictor of CVD, separately from its increase induced by renal dysfunction. Further, even in patients with previous IHD, low adiponectin may be a predictor of recurrent IHD. In the relative distribution of adiponectin isoforms, the percentage of the high-molecular-weight form in patients with severe CKD without IHD throughout the follow-up period was significantly higher than that in those who developed IHD.

Previous data from smaller studies have suggested that adiponectin levels are related to renal function in subjects with hypertension,[13] end-stage renal failure treated with hemodialysis therapy,[14] and renal diseases (including nephrotic syndrome).[15] Further, another recent report has suggested that renal dysfunction estimated by urea nitrogen may be the cause of hyperadiponectinemia in the elderly.[6] The present study extended these observations for adiponectin among the CKD stages of renal dysfunction, and multiple linear regression analysis clearly

Table 4
Adiponectin as a predictor of cardiovascular disease events

Variables, Unit of Increase	Crude		Adjusted CKD Stage		Adjusted Model*	
	HR (95% CI)	p Value	HR (95% CI)	p Value	HR (95% CI)	p Value
Adiponectin, 1 μg/ml increase	0.88 (0.79–0.97)	0.004	0.84 (0.72–0.94)	0.001	0.86 (0.75–0.96)	0.004
Smoker, yes	1.88 (1.14–3.24)	0.045	1.86 (1.12–3.21)	0.056	1.65 (0.98–2.89)	0.166
Previous IHD	1.61 (1.11–2.38)	0.011	1.59 (1.10–2.36)	0.014	1.49 (1.09–2.75)	0.041

* Adjusted by previous IHD, smoking, and CKD stages.
CI = confidence interval; HR = hazard ratio.

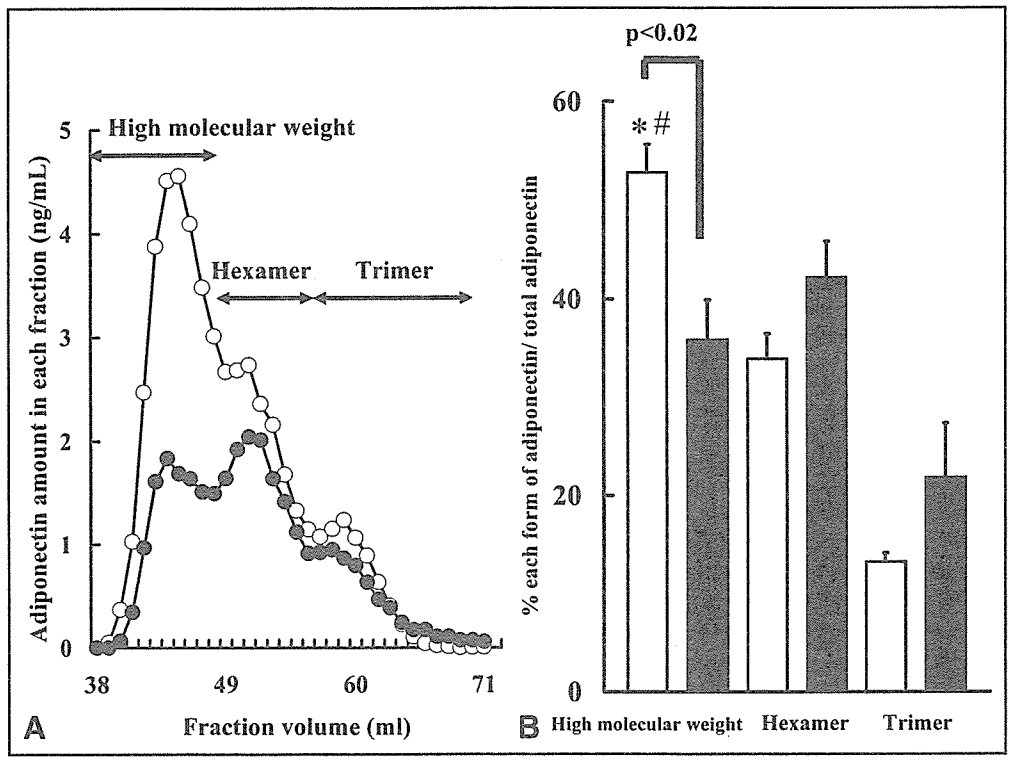

Figure 3. Oligometric state of adiponectin in human plasma from patients with severe CKD. *(A)* Averaged elution profiles of adiponectin in plasma from subjects without IHD (n = 4) *(white circles)* and those with IHD (n = 3) *(black circles)*. Plasma was fractionated by gel filtration chromatography, and the concentration of adiponectin in each 1-ml fraction was determined by enzyme-linked immunosorbent assay. *(B)* Percentage of each form of adiponectin in total adiponectin from subjects without IHD (n = 4) *(white bars)* and those with IHD (n = 3) *(black bars)*. Values are means ± SEs. $^{*}p < 0.05$ versus hexamer; $^{\#}p < 0.01$ versus trimer.

showed that CKD stages were independently associated with adiponectin concentration. Thus, our results also confirm that the kidney is the target organ regulating adiponectin concentration.

In this study, we carried out a prospective study by categorizing subjects into 2 groups according to median adiponectin concentration. Our results were partly in accordance with previous reports associating a higher adiponectin concentration with a lower risk of CVD in men[16] and in subjects with end-stage renal failure[14] and mild and moderate renal failure.[5] The present study showed that increased adiponectin was significantly associated with low risk of CVD events, even after adjustment for CKD stages, previous IHD, and smoking. In addition, even when the analysis was restricted to subjects with previous IHD, lower adiponectin was linked to a higher rate of recurrent IHD events. These results suggest that lower adiponectin concentration may be a potential risk factor for IHD, including recurrent IHD.

Previous reports have shown that high-molecular-weight adiponectin specifically confers the vasoprotective activities of this protein.[8] Our results lead to the notion that increased adiponectin in renal dysfunction, especially the high-molecular-weight isoform, may exert a protective role against CVD. However, further investigation is required to examine this hypothesis.

In conclusion, although renal function is a significant regulator of adiponectin, hypoadiponectinemia is a predictor of CVD, including recurrent IHD, apart from its increase induced by renal dysfunction.

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Association of genetic polymorphisms of *ACADSB* and *COMT* with human hypertension

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Objectives Genetically hypertensive rats provide an excellent model to investigate the genetic mechanisms of hypertension. We previously identified three differentially expressed genes, Acadsb (short/branched chain acyl-CoA dehydrogenase), Comt (catecholamine-*O*-methyltransferase), and Pnpo (pyridoxine 5′-phosphate oxidase), in hypertensive and normotensive rat kidneys as potential susceptibility genes for rat hypertension. We examined the association of human homologues of these genes with human hypertension.

Methods We sequenced three genes using samples from 48 or 96 hypertensive patients, identified single nucleotide polymorphisms, and genotyped them in a population-based sample of 1818 Japanese individuals (771 hypertensive individuals and 1047 controls).

Results After adjustments for age, body mass index, present illness (hyperlipidaemia, diabetes mellitus), and lifestyle (smoking, alcohol consumption), multivariate logistic regression analysis revealed that −512A>G in *ACADSB* was associated with hypertension in women (AA vs AG + GG: odds ratio = 0.70, 95% confidence interval = 0.53–0.94). This single nucleotide polymorphism was in tight linkage disequilibrium with −254G>A. Furthermore, −1187G>C in *COMT* was associated with hypertension in men (GG vs CG + CC: odds ratio = 0.69, 95% confidence interval = 0.52–0.93) and was in tight linkage disequilibrium with 186C>T. After adjustments described above, −512 A>G and −254G>A in *ACADSB* were associated with variations in systolic blood pressure. *ACADSB* was in tight linkage disequilibrium with *MGC35392* across a distance of 18.3 kb. *COMT* was not in linkage disequilibrium with any adjacent genes. Analysis indicated that two haplotypes of *COMT* were significantly associated with hypertension in men.

Conclusion Our study suggests the possible involvement of genetic polymorphisms in *ACADSB* and *COMT* in essential hypertension in the Japanese population.
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Keywords: catecholamine-*O*-methyltransferase, gene polymorphism, hypertension, salt sensitivity, short/branched-chain acyl-CoA dehydrogenase

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Conflict of interest: none

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Introduction

The identification of genes contributing to essential hypertension in humans is difficult because hypertension is a multifactorial disease resulting from both environmental and genetic factors. To overcome this difficulty and facilitate genetic analyses, genetically hypertensive rats such as spontaneously hypertensive rats and Dahl salt-sensitive (Dahl-S) rats have been utilized. Some genes that cause phenotypes such as hypertension and insulin resistance will be differentially expressed, and therefore candidates are sought from among genes found to be differentially expressed [1–3].

To identify candidate genes responsible for hypertension in Dahl-S rats, we previously utilized an oligonucleotide microarray analysis and identified differentially expressed genes in the kidneys of salt-loaded Dahl-S and Lewis rats [4]. To examine the association of these genes with variations in blood pressure, we obtained 101 F_2 males from Dahl-S and Lewis rats and performed precise blood pressure measurements by telemetric monitoring at 14 weeks of age following 9 weeks of salt loading. Correlation analyses of genotypes of 12 differentially expressed genes, and blood pressure variation in the F_2 rats, indicated that short/branched chain acyl-CoA dehydrogenase (*Acadsb*), catecholamine-*O*-methyltransferase (*Comt*), pyridoxine 5′-phosphate oxidase (*Pnpo*), and *Sah* (medium-chain acyl-CoA synthetase) showed a significant association with

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blood pressure variation. To extend these studies to hypertension in humans, it is important to know whether human homologues of these genes cause susceptibility to hypertension in humans.

The human chromosome is divided into discrete blocks, called haplotype blocks, separated by hot spots of recombination [5]. In the haplotype blocks, a small number of common haplotypes are present. The International HapMap Project was completed in 2005 and catalogued the patterns of more than 1 million single nucleotide polymorphisms (SNPs) [6]. It determined that most inter-SNP distances are less than 10 kb, although some are over 20 kb. Once a candidate polymorphism associated with a phenotype is identified, genotyping of SNPs in adjacent genes is highly important. If the haplotype block consists of multiple genes, the phenotype-causing SNP might be present in an adjacent gene.

In the present study, we attempted to evaluate three potential hypertension-causing genes, obtained from an earlier study in rats, using a population-based sample of 1818 Japanese (771 individuals with hypertension and 1047 controls). Since the *Sah* gene has already been studied extensively [7], we did not analyse it in here. We first identified genetic variations, primarily SNPs, in all the exons of three human homologues of the potential hypertension susceptibility genes, *ACADSB*, *COMT*, and *PNPO*. We next examined the association of the SNPs and their haplotypes of these candidate genes with the presence of hypertension and blood pressure variation in the general Japanese population. We also studied linkage disequilibrium at the candidate gene loci.

Methods

Participants

For the sequencing of DNA, patients with essential hypertension were recruited at the outpatient clinic of the Division of Hypertension and Nephrology, National Cardiovascular Center, Suita, Japan. For genotyping, 1818 individuals, including 771 patients with hypertension (396 men, 375 women) and 1047 controls (439 men, 608 women), were used as a population-based sample for the Suita study. The selection criteria and design of the Suita study have been described previously [8,9]. Only individuals who provided written informed consent for genetic analyses were included in this study, and the study protocol was approved by the Ethical Review Committee of the National Cardiovascular Center.

Measurements

Blood pressure measurements were taken after at least 10 min of rest in a sitting position. The recorded systolic and diastolic blood pressures were the means of two measurements recorded at least 3 min apart. Hypertension was defined as a systolic blood pressure (SBP) of at least 140 mmHg and/or a diastolic blood pressure (DBP) of at least 90 mmHg, or the current use of antihypertensive medication. Diabetes mellitus was defined as a fasting plasma glucose concentration greater than 7.0 mmol/l (126 mg/dl), a nonfasting plasma glucose concentration above 11.1 mmol/l (200 mg/dl), taking antidiabetic medication, or a HbA1c value of at least 6.5%. Hyperlipidaemia was defined as a total cholesterol concentration greater than 5.68 mmol/l (220 mg/dl) or the taking of antihyperlipidaemia medication.

Blood samples drawn from the participants after 12 h of fasting were collected in tubes containing ethylenediamine tetraacetic acid. We measured the total cholesterol and high-density lipoprotein-cholesterol levels with an autoanalyser (Toshiba TBA-80; Toshiba, Tokyo, Japan) in accordance with the Lipid Standardization Program of the US Centers for Disease Control and Prevention through the Osaka Medical Center for Health Science and Promotion, Japan.

Direct sequencing for single nucleotide polymorphism discovery, database searches for single nucleotide polymorphisms, and polymorphism genotyping

We sequenced the entire coding regions of three candidates for genes causing susceptibility to hypertension, *ACADSB*, *COMT*, and *PNPO*, in 48 or 96 hypertensive individuals in which we predicted the hypertension-susceptive SNPs would be found. Our methods for direct sequencing were described previously [10,11]. SNPs with a minor allele frequency of greater than 5% were considered candidates for genotyping using the TaqMan polymerase chain reaction system [12,13]. Since a missense mutation may cause direct susceptibility to hypertension, several missense mutations with a minor allele frequency of less than 5% were also genotyped. As a consequence, we genotyped five, seven, and two SNPs in *ACADSB*, *COMT*, and *PNPO*, respectively, from the general population.

The HapMap Project revealed that the inter-SNP distances in certain regions were greater than 20 kb [6]. Genotyping other polymorphisms in such a haplotype block is highly important. Within a region of 200 kb surrounding the *ACADSB* locus, 10 genes (*MGC45962*, *LOC118670*, *FLJ13490*, *MGC35392*, *PEGASUS*, *LOC340784*, *LOC387716*, *LOC387717*, *BUB3*, and *LOC390009*) are present. Seven genes (*TBX1*, *GNB1L*, *FL21125*, *TXNRD2*, *ARVCF*, *DKFZp761P1121*, and *DGCR8*) are located within approximately 200 kb of *COMT*. We determined SNPs in these genes using the database of Japanese Single Nucleotide Polymorphisms (http://snp.ims.u-tokyo.ac.jp/) [14,15] and genotyped the following 14 SNPs using the TaqMan polymerase chain reaction system: rs1891110-GA (*MGC45962*), rs3736583-AG (*MGC35392*), rs3736582-CG (*MGC35392*), rs11190-AC (*MGC35392*), rs752920-TA (*LOC390009*), rs2301558-CT (*TBX1*), rs2073767-CT

(*GNB1L*), rs1139793-GA (*TXNRD2*), rs1005873-AG (*TXNRD2*), rs2073747-GA (*ARVCF*), rs1990277-GA (*ARVCF*), rs1054215-CT (*DKFZp761P1121*), rs1640297-TC (*DGCR8*), and rs720012-AG (*DGCR8*).

Statistical analysis

Analysis of variance was used to compare mean values between groups and, if overall significance was demonstrated, the intergroup difference was assessed using a general linear model. Frequencies were compared using a chi-squared analysis.

The relationships between genotypes and the presence of hypertension were expressed in terms of odds ratios adjusted for several possible confounding effects, including age, body mass index, present illness (hyperlipidaemia and diabetes mellitus), and lifestyle choices (smoking and drinking). For multivariate risk predictors, the adjusted odds ratios were determined using 95% confidence intervals. For each gender, analysis of any association between genotype and blood pressure were also investigated using a logistic regression analysis that considered potential confounding risk variables, including age, body mass index, present illness (hyperlipidaemia and diabetes mellitus), lifestyle choices (smoking and alcohol consumption), and antihypertensive medication. All analyses were performed using SAS statistical software (release 6.12; SAS Institute Inc., Cary, North Carolina, USA) [16]. Linkage disequilibrium and haplotype analyses were conducted using SNPAlyze version 2.1 (DYNACOM Co., Ltd., Mohara, Japan). The pairwise linkage disequilibrium value, D', was obtained between the SNP and −512A>G at the *ACADSB* locus, and between the SNP and −1187G>C at the *COMT* locus. Haplotype frequencies were estimated from genotype data using an expectation maximization algorithm. Controlling for deviation from Hardy–Weinberg equilibrium gave nonsignificant results for all the SNPs examined in the current study.

Results

General characteristics of study participants

The characteristics of the 1818 individuals (835 men and 983 women) are summarized in Table 1. Age, SBP, DBP, body mass index, percentages of current smokers and drinkers, prevalence of hypertension, and prevalence of diabetes mellitus were significantly higher in the men than in the women. Total cholesterol, high-density lipoprotein-cholesterol, and the percentage of hyperlipidaemic patients were significantly higher in the women than in the men.

Polymorphisms in *ACADSB*, *COMT*, and *PNPO*, and single nucleotide polymorphism genotyping

We sequenced either 96 or 182 alleles from 48 or 96 Japanese hypertensive patients for the *ACADSB*, *COMT*, and *PNPO* genes, and identified 14, 14, and five polymorphisms, respectively (Table 2). There were two and three missense mutations in *ACADSB* and *COMT*, respectively. The R13K mutation in *ACADSB* and the A72S and V158M mutations in *COMT* were common, with minor allele frequencies of 0.125, 0.093, and 0.279, respectively. The V158M mutation in *COMT* is known to be functional; the enzyme containing Met has one-quarter the activity of the Val-containing enzyme [17]. The H31R mutation in *ACADSB* showed a minor allele frequency of 0.021, and the K212T mutation in *COMT* showed a minor allele frequency of 0.005. Considering the allele frequencies and linkage disequilibrium, we selected five, seven, and two SNPs in *ACADSB*, *COMT*, and *PNPO*, respectively, and genotyped them using large-scale population-based samples.

Table 1 **Basic characteristics of the participants**

Characteristic	Women ($n=983$)	Men ($n=835$)
Age (years)	63.3 ± 11.0	66.3 ± 11.1*
Systolic blood pressure (mmHg)	128.0 ± 19.6	131.9 ± 19.5*
Diastolic blood pressure (mmHg)	76.6 ± 9.8	79.7 ± 10.7*
Body mass index (kg/m^2)	22.3 ± 3.2	23.3 ± 3.0*
Total cholesterol (mmol/l)	5.57 ± 0.79*	5.10 ± 0.78
High-density lipoprotein-cholesterol (mmol/l)	1.67 ± 0.40*	1.42 ± 0.36
Current smokers (%)	6.3	30.1†
Current drinkers (%)	29.3	67.0†
Present illness (%)		
Hypertension	38.2	47.4†
Hyperlipidaemia	55.2†	27.4
Diabetes mellitus	5.2	12.6†

Values presented as the mean ± SD or the percentage. The indications for each condition were as follows: hypertension, systolic blood pressure ≥ 140 mmHg and/or diastolic blood pressure ≥ 90 mmHg, or antihypertensive medication; hyperlipidaemia, total cholesterol ≥ 5.68 mmol/l (220 mg/dl) or antihyperlipidaemia medication; and diabetes, fasting plasma glucose ≥ 7.0 mmol/l (126 mg/dl), nonfasting plasma glucose ≥ 11.1 mmol/l (200 mg/dl), or antidiabetic medication. *$P < 0.05$ between females and males with Student's t-test. †$P < 0.05$ between females and males with a chi-squared test.

Association of single nucleotide polymorphisms with hypertension

Multivariate logistic regression analysis, after adjustments for age, body mass index, current illness (hyperlipidaemia and diabetes mellitus), and lifestyle (smoking and alcohol consumption), revealed that −512A>G and −254G>A in *ACADSB* in tight linkage disequilibrium showed an association with the presence of hypertension in women (−512A>G: AA vs AG + GG: odds ratio = 0.70, 95% confidence interval = 0.53–0.94, $P = 0.0163$; −254G>A: GG vs GA + AA, odds ratio = 0.70, 95% confidence interval = 0.53–0.94, $P = 0.0171$) (Table 3). In addition, −1187G>C and 186C>T in *COMT* in tight linkage disequilibrium were associated with hypertension in men (−1187G>C: GG vs GC + CC, odds ratio = 0.69, 95% confidence interval = 0.52–0.93, $P = 0.0122$; 186C>T: CC vs CT + TT, odds ratio = 0.69, 0.69, 95% confidence interval = 0.52–0.92, $P = 0.0116$) (Table 3). A functional SNP in *COMT*, 1222G>A, accompanied by the V158M substitution, was marginally associated with hypertension ($P = 0.0742$).

Table 2 List of polymorphisms and their allele frequencies in *ACADSB*, *COMT*, and *PNPO*, as identified by direct sequencing

				Allele frequency				
Single nucleotide polymorphism	LD	Amino acid change	Region	Allele 1	Allele 2	Flanking sequence	Taqman	dbSNP ID
ACADSB								
−512A>G	a		Promoter	0.714	0.286	ccctccggctaa[a/g]gaggtcccgggc	Taqman	rs2277249
−254G>A	a		Promoter	0.714	0.286	accgtcacagtc[g/a]ccgccgccatct	Taqman	rs2277250
−211C>A			Promoter	0.995	0.005	ccttcccgcccc[c/a]ctgccttgctca		
−107G>A	b		Promoter	0.979	0.021	gcagggattaag[g/a]gggggtgtgtgc		
−80G>C			Promoter	0.995	0.005	ggcgggtactga[g/c]tgggcggggcct		
−22A>G			Promoter	0.995	0.005	ccagaggcgcag[a/g]gcggagaggcct		
38G>A		R13K	Exon 1	0.875	0.125	TGCGCGGCAGCA[G/A]GCTGGTGAGTGC	Taqman	
89delG			Intron 1	0.995	0.005	agggcgaccttg[g/-]cccctggaatcg		
25376A>G	b	H31R	Exon 2	0.979	0.021	AGATTCCTCCTC[A/G]TGTCTCAAAATC	Taqman	
31341delTAA	c		Intron 3	0.196	0.804	aaataataataa[taa/-]atatggttacag		
31379G>A			Intron 3	0.989	0.011	ttgttcatgcaa[g/a]aaatttccccat		
32308C>T		H213H	Exon 5	0.896	0.104	CAGTGCTGAGCA[C/T]GCAGGGCTCTTT		
43942A>G	c		Intron 9	0.198	0.802	gccactaacagt[a/g]aatccatgttgc	Taqman	rs2421166
44814C>T			3′-UTR	0.979	0.021	TGGGAGTAAGTG[C/T]CTTGCGTGGGAA		
COMT								
−20878A>G			Promoter	0.990	0.010	accctcacgagg[a/g]caccccggccgc		
−20531G>A			Intron 1	0.984	0.016	gtggggaattcg[g/a]accgctgtgaag		
−1187G>C	d		Intron 2	0.724	0.276	ggtacagattcc[g/c]gcccggtgcatg	Taqman	rs165656
−98A>G	e		Intron 2	0.728	0.272	ttgcccctctgc[a/g]aacacaaggggg		rs6269
186C>T	d	H62H	Exon 3	0.717	0.283	CATCCTGAACCA[C/T]GTGCTGCAGCAT	Taqman	rs4633
214G>T		A72S	Exon 3	0.907	0.093	GAGCCCGGGAAC[G/T]CACAGAGCGTGC	Taqman	rs6267
379A>G	e		Intron 3	0.725	0.275	tgttatcacccc[a/g]tttccagggggc		rs2239393
971G>A			Intron 3	0.995	0.005	aggtgggggcc[g/a]tgcctggggatc		
1158C>G	e	L136L	Exon 4	0.716	0.284	AGGGGCGAGGCT[C/G]ATCACCATCGAG	Taqman	rs4818
1222G>A	d	V158M	Exon 4	0.721	0.279	GATTTCGCTGGC[G/A]TGAAGGACAAGg	Taqman	rs4680
1755G>A		P199P	Exon 5	0.941	0.059	CCGGTACCTGCC[G/A]GACACGCTTCTC		rs769224
1848G>C			Intron 5	0.856	0.144	agcctctccaaa[g/c]agccaggcattc	Taqman	rs4646315
6029A>C		K212T	Exon 6	0.995	0.005	GCCTGCTGCGGA[A/C]GGGGACAGTGCT		
6220−6221insC			3′-UTR	0.468	0.532	GACTGCCCCCCC[-/C]GGCCCCCCTCTC	Taqman	rs362204
PNPO								
−139A>C			Promoter	0.989	0.011	ttggctccgagg[a/c]cttaggacctgt		
1657C>T		S55S	Exon 2	0.840	0.160	TCATCTGACCTC[C/T]CTTGACCCAGTG	Taqman	
3848C>T			Intron 3	0.379	0.621	tcctctccctgt[c/t]ctgatggctggc	Taqman	rs4491575
4119G>A			Intron 4	0.995	0.005	acagagaggaac[g/a]gggcctgtgctg		
4308T>C		D180D	Exon 5	0.995	0.005	TGTGATCCCTGA[T/C]CGGGAGgtgagt		

ACADSB, acyl-Coenzyme A dehydrogenase, short/branched chain (10q25-q26); *COMT*, catechol-*O*-methyltransferase (22q11.2); *PNPO*, pyridoxine-5-prime-phosphate oxidase (17); UTR, untranslated region. The apparent linkage disequilibrium (LD), defined by $r^2 > 0.5$, is indicated by 'a−e' in the LD column. Single nucleotide polymorphisms for large-scale genotyping are indicated by 'Taqman'. The A of the ATG of the initiating Met codon is denoted nucelotide + 1, following recommendations by the Nomenclature Working Group [29]. Localization of the human chromosome is shown in parentheses. The nucleotide sequences (GenBank accession number NT_030059.12 for *ACADSB*, NT_011519.10 for *COMT*, and NT_010783.14 for *PNPO*) were used as reference sequences. Uppercase and lowercase letters in the flanking sequences are sequences in extron and intron regions, respectively.

Table 3 Odds ratio of polymorphisms in *COMT* and *ACADSB*

			Women		Men	
Gene	SNPs (allele frequency)	Genotype	Odds ratio (95% confidence interval)*	*P* value	Odds ratio (95% confidence interval)*	*P* value
ACADSB	−512A>G[b]	AA	1	0.0163	1	0.3832
	(0.738/0.262)	AG + GG	0.70 (0.53−0.94)		1.13 (0.85−1.51)	
		AA + AG	1	0.5695	1	0.4850
		GG	0.84 (0.46−1.54)		1.21 (0.71−2.07)	
ACADSB	−254G>A[b]	GG	1	0.0171	1	0.3785
	(0.738/0.262)	GA + AA	0.70 (0.53−0.94)		1.14 (0.86−1.51)	
		GG + GA	1	0.5676	1	0.3899
		AA	0.84 (0.46−1.54)		1.27 (0.74−2.18)	
COMT	−1187G>C[a]	GG	1	0.2791	1	0.0122
	(0.703/0.297)	GC + CC	1.18 (0.88−1.56)		0.69 (0.52−0.93)	
		GG + GC	1	0.6844	1	0.1573
		CC	0.89 (0.52−1.54)		0.70 (0.43−1.15)	
COMT	186C>T[a]	CC	1	0.3097	1	0.0116
	(0.704/0.296)	CT + TT	1.16 (0.87−1.54)		0.69 (0.52−0.92)	
		CC + CT	1	0.4891	1	0.1555
		TT	0.83 (0.48−1.43)		0.70 (0.43−1.15)	
COMT	1222G>A[a]	GG	1	0.1522	1	0.0742
	(0.695/0.305)	GA + AA	1.23 (0.92−1.64)		0.77 (0.58−1.03)	
		GG + GA	1	0.4946	1	0.4935
		AA	0.83 (0.50−1.41)		0.85 (0.52−1.37)	

* Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidaemia and diabetes mellitus), and lifestyle (smoking and drinking). The apparent linkage disequilibrium, defined by $r^2 > 0.5$, is indicated by 'a' and 'b' in the single nucleotide polymorphisms (SNPs) column.

Table 4 Association of genotypes with blood pressure variation

Gene	Single nucleotide polymorphism	Allele 1/2 (allele frequency)	Sex	BP	Genotype group	BP, mean ± SD (mmHg)	*P* value*	Variation of mean BP (mmHg)
ACADSB	−512A>G[a]	A/G (0.738/0.262)	Women	SBP	AA AG + GG	128.77 ± 0.69 126.48 ± 0.80	0.0302	2.29
ACADSB	−254G>A[a]	G/A (0.738/0.262)	Women	SBP	GG GA + AA	128.82 ± 0.69 126.47 ± 0.79	0.0264	2.35
ACADSB	38G>A (Arg13Lys)	G/A (0.878/0.122)	Women	DBP	GG + GA AA	76.46 ± 0.30 82.37 ± 2.59	0.0235	5.91

BP, blood pressure; SBP, systolic blood pressure; DBP, diastolic blood pressure. [a] The apparent linkage disequilibrium, defined by $r^2 > 0.5$. * Conditional logistic analysis, adjusted for age, body mass index, present illness (hyperlipidaemia and diabetes mellitus), and lifestyle (smoking and drinking).

SBP was 2.29 mmHg higher in women with the *ACADSB* AA genotype −512A>G than women with the AG + GG genotype ($P = 0.030$), and 2.35 mmHg higher in women with the *ACADSB* GG genotype −254G>A than women with the GA + AA genotype ($P = 0.026$), after adjusting for the factors described above (Table 4). In addition, DBP was 5.90 mmHg higher in women with the *ACADSB* GG + GA genotype 38G>A than women with the AA genotype ($P = 0.024$) (Table 4). This SNP results in the amino acid substitution R13K and appears to be of functional significance.

Table 5 presents the results of the analysis of haplotype frequency for the SNPs of these three genes between hypertensive individuals and normotensive individuals. We identified haplotypes three and seven of *COMT* as having a significantly lower ($P = 0.006$) and higher frequency ($P = 0.029$) in hypertensive men than in normotensive men, respectively.

Taken together, *ACADSB* was associated with both hypertension and blood pressure variation, and *COMT* was associated with hypertension.

Linkage disequilibrium of *ACADSB* and *COMT* with adjacent genes

It is possible that the polymorphisms in *ACADSB* and *COMT* that are significantly associated with hypertension are in linkage disequilibrium with other genes in their vicinities and compose a haplotype block. To evaluate the haplotype block structure in these regions, we genotyped 14 additional SNPs present within approximately 200 kb. The pairwise linkage disequilibrium parameters, D', calculated from the genotyping data are shown in Fig. 1. These methods revealed that at the *ACADSB* locus, IMS-JST080977 in *MGC35392*, which is 18.3 kb from −512A>G in *ACADSB*, exhibited a D' value of 0.997, while IMS-JST080979 in *MGC35392*, which is 25.2 kb from −512A>G in *ACADSB*, showed a D' value of 0.928, indicating a large haplotype block at this locus. The haplotype structure of the *ACADSB* locus suggests the association of this block with the presence of hypertension. *COMT*, on the other hand, was not in linkage disequilibrium with any adjacent genes.

Discussion

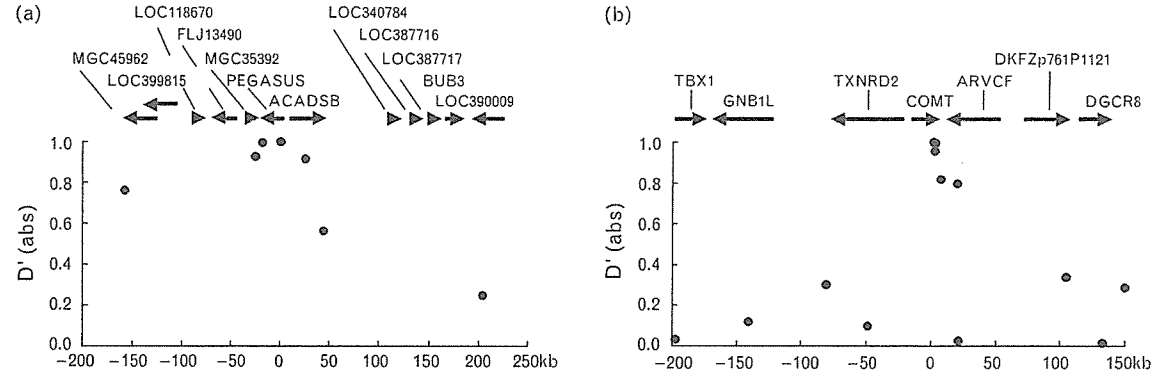
We previously identified differentially expressed genes in the kidneys of salt-loaded Dahl-S and Lewis rats [4].

Table 5 Haplotype frequency of *COMT*, *ACADSB*, and *PNPO* genes in hypertensive individuals (HT) and normotensive individuals (NT)

Gene	Haplotype		Men (%)				Women (%)			
			HT (812 alleles)	NT (902 alleles)	χ^2	*P*	HT (772 alleles)	NT (1242 alleles)	χ^2	*P*
COMT		−1187/186/214/1158/1222/1848/6221insC								
	1	G/C/G/C/G/G/−	22.8	23.6	0.166	0.684	20.9	21.7	0.184	0.668
	2	G/C/G/G/G/G/C	20.1	18.4	0.768	0.381	21.6	21.3	0.040	0.842
	3	C/T/G/C/A/G/C	12.4	17.2	7.638	0.006	14.9	15.1	0.022	0.883
	4	C/T/G/C/A/C/C	12.2	12.4	0.020	0.888	14.0	11.8	1.977	0.160
	5	G/C/G/G/G/G/−	9.5	9.5	0.001	0.971	11.3	9.5	1.611	0.204
	6	G/C/T/C/G/G/−	10.2	8.3	1.854	0.173	7.5	8.3	0.397	0.529
	7	G/C/G/C/G/G/C	9.0	6.2	4.748	0.029	6.1	8.0	2.565	0.109
	8	G/C/G/C/A/G/−	1.7	1.3	0.443	0.506	1.2	1.5	0.059	0.809
ACADSB		−512/38/25376/43942								
	1	A/G/A/G	63.5	65.5	0.762	0.383	69.6	66.3	2.488	0.125
	2	G/G/A/A	15.1	13.1	1.426	0.232	10.3	12.7	2.646	0.104
	3	G/A/A/G	13.0	12.0	0.406	0.524	11.0	12.5	1.030	0.310
	4	A/G/A/A	5.5	7.1	1.684	0.194	6.3	6.7	0.097	0.756
	5	A/G/G/G	1.4	0.7	2.110	0.146	1.9	1.0	2.678	0.102
	6	G/G/A/G	1.2	1.4	0.135	0.713	0.8	0.8	0.016	0.898
PNPO		1657/4308								
	1	C/T	60.3	61.1	0.139	0.709	59.5	59.3	0.015	0.904
	2	C/C	22.9	22.0	0.199	0.656	24.7	23.8	0.231	0.631
	3	T/C	16.6	16.5	0.010	0.920	15.8	16.9	0.449	0.503

Haplotypes with frequency ≥ 1.0% are shown.

Fig. 1



Pairwise linkage disequilibrium at the *ACADSB* (a) and *COMT* (b) loci. The pairwise linkage disequilibrium value, *D'*, was obtained between the single nucleotide polymorphism and −512A>G at the *ACADSB* locus, and between the single nucleotide polymorphism and −1187G>C at the *COMT* locus.

In these experiments, we obtained 101 F_2 male rats from Dahl-S and Lewis rats and performed precise measurements of blood pressure by telemetric monitoring at 14 weeks of age, following 9 weeks of salt loading. Correlation analyses of the genotypes of 12 differentially expressed genes and the variations in blood pressure in F_2 rats indicated that *Acadsb*, *Comt*, *Pnpo*, and *Sah* are significantly associated with blood pressure. In the current study, we have examined 1818 individuals for a relationship between the genes, *ACADSB*, *COMT*, and *PNPO*, and hypertension or blood pressure variation. These three genes were originally selected based on studies in the Dahl-S rat. We determined that two SNPs in *ACADSB*, −512A>G and −254G>A, which are in tight linkage disequilibrium, were associated with both hypertension and blood pressure variation. Two SNPs in *COMT*, −1187G>C and 186C>T, which are also in tight linkage disequilibrium, were associated with hypertension. These candidate genes were selected from the salt-loaded rats, and therefore the genetic association of these genes with hypertension might be greater if we had selected patients with salt-sensitive hypertension.

In this study, we genotyped 14 SNPs in total; therefore, after applying the Bonferroni correction for multiple testing, the level of significance was $P < 0.004$ (0.05/14 for 14 loci). Unfortunately, none of the SNPs appeared to be significant with the use of a strict Bonferroni correction. As described, however, two SNPs in *ACADSB* were associated with both hypertension and blood pressure variation. In addition, one SNP and two haplotypes in *COMT* were significantly associated with hypertension. These two genes were therefore considered valid as hypertensive candidates.

This study was undertaken to prove that candidate susceptibility genes for hypertension in the Dahl-S rat studies might also be applicable to humans. The genes *Acadsb* and *Comt* were associated with hypertension in humans, but *Pnpo* was not. *Sah* was the first example of a possible link between a differentially expressed gene in rats and human hypertension [7]. Our study is another example linking candidate susceptibility genes for hypertension identified in rats, to humans, and it also revealed genetic differences between humans and rats, particularly in salt-loaded Dahl-S rats, in terms of sensitivity to hypertension. The population of F_2 rats and the general population in this study may not be large enough to provide good statistical power. As stated above, when a human study is performed using a subgroup of salt-sensitive patients, stronger associations may become apparent.

ACADSB, short/branched chain acyl-CoA dehydrogenase, is a member of the acyl-CoA dehydrogenase family. Acyl-CoA dehydrogenases with specificity for different chain-lengths of fatty acids carry out the first step of β-oxidation in the mitochondria, each round of which removes two-carbon units as acetyl-CoA for entry into the tricarboxylic acid cycle. Acyl-CoA dehydrogenases are mitochondrial enzymes involved in the metabolism of fatty acids and branched-chain amino acids, which are required to meet physiologic energy requirements during illness and periods of fasting or under physiologic stress. In addition, two other important kidney-specific genes involved in fatty acid metabolism, *SAH* and *KS* (kidney specific) have acyl-CoA synthetase activity for medium-chain fatty acids. Both genes were isolated by differential screening from a genetically hypertensive rat strain, the spontaneously hypertensive rat [1,7,18]. Moreover, polymorphism of *SAH* was associated with cardiovascular diseases, including hypertension, hypertriglyceridaemia, hypercholesterolemia, and obesity [7]. Both *ACADSB* and *SAH* are therefore related to fatty acid metabolism and their products may exhibit some link or cross-talk that could be involved in hypertension.

Human *ACADSB* is located at 10q25-26, which corresponds to 1q35 in rats. This rat locus is reportedly related to hypertension [19], and the genomic structure of *ACADSB* indicates that *ACADSB* is located close to *PEGASUS* in a head-to-head fashion (Fig. 1). Two SNPs in *ACADSB*, −512A>G and −254G>A, which are both associated with hypertension and blood pressure variation, correspond to −9893T>C in intron 1 and −10151C>T in the 5′-untranslated region of *PEGASUS*, respectively. In searching for a transcription factor-binding motif, we determined that the nucleotide change −254G>A would give rise to the AP-1 transcription factor-binding motif. PEGASUS is a member of the Ikaros family of transcription factors, and is expressed not only in haematopoietic cell lines, as are other Ikaros family members, but also in other tissues, including the brain, heart, skeletal muscle, kidney, and liver [20]. The PEGASUS study is highly limited, and no direct links between *PEGASUS* and blood pressure have been reported. Taken together, we consider *ACADSB/PEGASUS* to be a susceptibility gene for hypertension.

COMT is a ubiquitous enzyme that catalyses the transfer of a methyl group from *S*-adenosylmethionine to catecholamines. The substrates of COMT are catechol neurotransmitters (e.g. dopamine, epinephrine, and norepinephrine), catechol estrogens (e.g. carcinogenic 4-hydroxyestradiol), indolic intermediates in melanin metabolism, xenobiotic catechols (e.g. carcinogenic flavonoids), and drugs (e.g. levodopa). COMT therefore plays an important role in the pathophysiology of Parkinson's disease, depression, oestrogen-induced cancers, and hypertension [21]. A recent study indicated that *Comt* gene-disrupted mice showed resistance to salt-induced hypertension, and the sodium-induced increase in blood pressure in wild-type mice was completely normalized by treatment with the COMT inhibitor nitecapone [22]. At baseline, 24-h urinary excretion of dopamine was increased in *Comt*-deficient mice compared with wild-type mice. In *Comt*-deficient and wild-type mice, a high-sodium diet increased urinary dopamine excretion by 405 and 660% (reflected as 102 and 212% increases in dopamine excretion), respectively. COMT can therefore regulate blood pressure, sodium excretion, and renal dopaminergic tone [22].

A functional polymorphism, 1222G>A, encoding V158M, has been reported in *COMT*. The enzyme containing Met is unstable at 37°C and has one-quarter the activity of the Val-containing enzyme [17]. In the present study, the allele frequencies of 1222G>A were 0.695 and 0.305, respectively ($n = 1818$) (Table 3). This functional SNP showed marginal significance in the case–control setting (Table 3), and it also showed linkage disequilibrium with −1187G>C and 186C>T in *COMT* (Table 2). A recent study showed that this SNP was associated with myocardial infarction in a hypertensive population, in which the low activity *COMT* genotype is protective against myocardial infarction [23].

In summary, we have studied the association between the presence of hypertension or variation in blood pressure and candidate genes selected based on experiments with the Dahl-S hypertensive rat previously reported by our group [4]. *ACADSB/PEGASUS* was associated with both hypertension and blood pressure variation, and *COMT* was associated with hypertension. Due to false positives, false negatives, and true variability between different populations, association studies are not consistently reproducible [24]. Confirmation of these results using additional cohorts is therefore required.

Perspective

Since essential hypertension is a multifactorial disease, genetic influence is thought to play an important role in its initial stages and progression. Multiple approaches have been used to detect causative genetic polymorphisms [25–28]. The candidate gene approach is the most popular method, but crucial genetic polymorphisms are still only poorly understood. We therefore attempted to identify genetic polymorphisms that cause susceptibility to hypertension on the basis of the results of expression studies previously performed in a hypertensive rat model. We revealed that two SNPs in *ACADSB/PEGASUS* and SNPs of *COMT* might cause susceptibility to essential hypertension. These results were obtained from one population. Further replication of these results in an independent population is therefore necessary. Although functional analyses are needed to clarify the association of these SNPs with the pathogenesis of hypertension, we plan to apply this information in a gene evaluation system that will develop individualized treatment for hypertension.

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サイアザイド系利尿薬の降圧効果に関与する遺伝子多型*

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はじめに

ヒトゲノム・シークエンスの完了を受け，これまでわからなかった薬剤の作用部位や詳細な分子構造が明らかにされていくにつれ，それらを標的とした薬理ゲノミクス（pharmacogenomics）が急速に進歩してきた。この分野が進むことにより，遺伝子情報を基に降圧薬を選択する高血圧の個別化診療を行うことが可能になることが期待されている。われわれは2005年3月に終了したミレニアム・ゲノム・プロジェクト（MGP）において高血圧関連遺伝子解析を担当し，特に降圧薬の効果にかかわる遺伝子多型の探索に力を入れてきた[1,2]。本稿ではMGPにおけるわれわれの成果をふまえ，特に遺伝的な素因が薬理効果に大きく関与するとされるサイアザイド系利尿薬のpharmacogenomicsにつき概説する。

Ⅰ．サイアザイド系利尿薬関連遺伝子多型（海外からの報告）

サイアザイド系利尿薬の効果に関与する遺伝子多型にはいくつかの報告がある。具体的にはG蛋白β3サブユニット遺伝子（*GNB3*）C825T多型(3)，α-Adducin遺伝子（*ADD1*）Gly460Trp多型(4)，レニン・アンジオテンシン系（RAS）遺伝子のACE遺伝子（*ACE*）Ⅰ/D多型(5)，ならびにアフリカ系アメリカ人の女性ではアンジオテンシンⅡ1型受容体遺伝子（*AT1R*）A1166C多型やアンジオテンシノゲン（*AGT*）遺伝子G-6A多型(6)，さらにはeNOS遺伝子Glu298Asp多型(7)でも報告されている。

*GNB3*のC825T多型はβ3-shortを生じ，高血圧の原因遺伝子変異の一つとも考えられている。*GNB3*は12p13領域に位置し，その第10エクソン上にC825Tは存在する。C825Tはサイレントな変異であるが，スプライシングの異常を生じその結果，第8，9エクソンの一部に対応する41残基を欠失するβ3-shortを産生する確率を上げると推定されている。β3-shortで欠失する部分はG蛋白αサブユニット（Gα）との相互作用に重要な場所に位置しているため，受容体によるGαの活性化を促進すると考えられている。この*GNB3*のC825T多型が低レニン活性と関連することが報告されたため，サイアザイド系利尿薬の効果にも影響することが予測され197人のアフリカ系アメリカ人と190人の白人で検討された結果，CC，CT，TTの順に有意にサイアザイド系利尿薬による降圧効果が良好であった[3]。

Adducinは細胞膜骨格蛋白でαβのヘテロ二量体を形成する。Milan高血圧ラットの解析でαとβadducin遺伝子のミスセンス変異が腎臓でのNa再吸収亢進に関与し，高血圧を呈することか

* Gene polymorphisms related to the effectiveness of thiazide diuretics
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表　サイアザイド系利尿薬感受性遺伝子多型における多型間の反応・非反応患者の頻度差

多型	性	遺伝型	反応群	非反応群		
TSC, C1784T	男女	CC	40	20	χ^2=6.052	P=0.049
		CT	5	9		
		TT	0	1		
		CC	40	20	χ^2=5.556	P=0.037
		TT+CT	5	10		
	オッズ比=4.000		95%信頼区間=1.204～13.284			
		C allele	85	49	χ^2=6.168	P=0.016
		T allele	5	11		
	オッズ比=3.816		95%信頼区間=1.253～11.627			
ADRB3, T727C	男女	CC	1	1	χ^2=10.649	P=0.005
		CT	3	11		
		TT	40	18		
		TT	40	18	χ^2=10.056	P=0.003
		CC+CT	4	12		
	オッズ比=6.667		95%信頼区間=1.889～23.525			
		C allele	5	13	χ^2=8.533	P=0.005
		T allele	83	47		
	オッズ比=4.591		95%信頼区間=1.541～13.680			

高血圧患者で新規にサイアザイド系利尿薬が処方された患者を対象に服用開始前後3回の外来血圧を平均し，平均血圧で5 mmHg以上の降圧を認めた群を反応群，それ未満もしくは投与後血圧が上昇した群を非反応群と定義した。（文献9）より引用）

ら，ヒトにおいても*ADD1*の遺伝子多型と高血圧との関連が検討され，Gly460Trp多型で有意な関係が認められた。日本人でも低レニン性高血圧には有意な関連を示すため，食塩感受性に影響を及ぼしているものと考えられるが，Glorioso らのイタリア人における検討では，高血圧・低レニンの多いTrp460アレルの保有者ではサイアザイド系利尿薬への反応性が良好であった[4]。さらに同グループは*ACE* I/D多型と*ADD1* Gly460Trp多型を組み合わせた場合，単独よりもサイアザイド系利尿薬への反応性を予測できると報告した。つまり，サイアザイド系利尿薬への感受性が強い*ADD1* Trp 460型と*ACE* I型の両方を有する群で最もサイアザイド系利尿薬投与後の降圧効果が良好であった[8]。これらの成績は欧米からのもので，日本人である程度大規模なサイアザイド系利尿薬の効果に関連する遺伝子変異・多型の報告はなされていなかった。

II．サイアザイド系利尿薬関連遺伝子多型（わが国からの報告）

われわれは，76人の新規にサイアザイド系利尿薬を服用した患者の降圧効果から感受性遺伝子多型の同定を後向きの解析手法にて試みた[9]。国立循環器病センター高血圧腎臓内科外来受診中の高血圧患者で新規にサイアザイド系利尿薬が処方された患者を対象に，服用開始前後3回の外来血圧を平均し，平均血圧で5 mmHg以上の降圧を認めた群を反応群，それ未満もしくは投与後血圧が上昇した群を非反応群と定義した。検討した遺伝子多型は*GNB3* C825T, *ADD1* Gly460Trp, RASや交感神経系（SNS）関連遺伝子に加え，サイアザイド感受性Na-Cl共輸送体遺伝子（*TSC*），サイアザイド系利尿薬感受性のGordon症候群の原因遺伝子である*WNK1*，*WNK4*，ミネラルコルチコイ