clear what plasma BNP levels reflect and whether previous observations in patients without CKD could be applied to ESRD patients.

Accordingly, we tested the hypothesis that plasma BNP levels might reflect the presence and severity of stable CAD in chronic HD patients. We also examined left ventricular end-diastolic wall stress (LV EDWS), which we previously found is a crucial haemodynamic determinant of plasma BNP levels [7], to clarify the contribution of haemodynamic factors in the regulation of plasma BNP in this setting.

Subjects and methods

Study patients

One hundred seventy-nine chronic HD patients who had been referred for CAG due to symptoms of angina pectoris, or with objective evidence of ischaemia (positive exercise electrocardiogram or nuclear test), were enrolled in the present study. Patients with ACS including unstable angina and acute myocardial infarction were excluded from this study. All patients underwent regular 4-h sessions of HD using polysulfone membrane filters three times weekly. After dialysis, they were shown to have a condition that entailed no clinical signs of hypervolaemia such as oedema, dyspnea or an excessive increase in arterial blood pressure, which was established under the supervision of experienced nephrologists.

Plasma BNP levels, haemoglobin, serum albumin, serum C-reactive protein (CRP) and serum creatinine were determined in blood samples withdrawn immediately before coronary angiography (CAG). Echocardiographic examination was performed after an HD session on the day before CAG.

CAG and lesion morphology

CAG was performed following a standard technique. Two experienced cardiologists who were blinded to plasma BNP levels assessed the coronary angiograms. If they disagreed, a third expert examined the angiogram to determine the characteristics of the lesions. Diameter stenosis of $\geq$70% by quantitative angiography was accepted as significant. Extension of CAD was classified as 1-, 2- or 3-vessel disease (VD) by the standard method. We also estimated the degree of CAD using the Gensini score and the CAD prognostic index. The former is a measure of the extent and severity of CAD and is computed by assigning a severity score to each coronary segment according to the degree of luminal narrowing and its geographic importance [19]. The latter considers the number of diseased vessels, the presence of left anterior descending or left main coronary disease, which have been validated in an overlapping heart failure population [20,21]. Left ventricular pressure was recorded with a 5-F pigtail catheter connected to a fluid-filled transducer. Left ventricular volume and ejection fraction (EF) were determined by left ventriculography with a contrast medium using Kennedy's formula.

Echocardiography

Echocardiographic examinations were performed in all patients with a Sonos 5500 machine equipped with a 2.5 MHz probe. M-mode images were obtained to measure left atrial and ventricular dimensions [22]. The left ventricular mass index (LVMI) was estimated using the formula of Devereux *et al.* In patients with sinus rhythm, the pulsed Doppler transmitral flow velocity was recorded to measure the ratio of peak mitral E-wave velocity to peak mitral A-wave velocity (E/A ratio) and the deceleration time of the mitral E-wave velocity. Based on haemodynamic and echocardiographic data, end-diastolic and systolic meridional WS were calculated as follows: $WS = 0.334 \times P(LVID)/WT(1 + WT/LVID)$, where P = LV pressure (i.e. peak systolic pressure or end-diastolic pressure (EDP), which was obtained during cardiac catheterization), LVID = left ventricular internal dimension and WT = wall thickness [7].

Statistical analysis

Groups were compared using a chi-square analysis for proportions and unpaired Student t tests for continuous variables. Cut-off levels of BNP and the sensitivities and specificities of the cut-off levels were calculated using a receiver operating characteristics (ROC) curve analysis. The linearity of a relationship between two variables was assessed by linear regression analysis. Further multivariable analysis was performed to evaluate the independent relationship between severity of CAD (VD, Gensini score or CAD prognostic index) and plasma BNP levels in concert with demographic variables, haemodynamic indexes and laboratory data using JMP version 5.0. Variables included in the analysis were sex, age, BMI (body mass index), NYHA (New York Heart Association) class, HT (hypertension), DM (diabetes mellitus), HLP (hyperlipidaemia), AF (atrial fibrillation), HD etiology and duration, medications, haemodynamic and echocardiographic indexes and laboratory data (creatinine, CRP, albumin and haemoglobin); $P < 0.05$ was considered significant. Results were expressed as mean $\pm$ SEM.

Results

Patient characteristics

The baseline clinical characteristics in chronic HD patients according to the presence and extent of CAD are shown in Tables 1 and 2, respectively. In all of the studied patients, the mean age was 67.6 $\pm$ 0.7 years and 13% of the patients were female. Patients with CAD were more likely to have a history of DM and HLP. There were no significant differences in other past history, duration of HD, etiology, medications or haemoglobin and creatinine levels between CAD and non-CAD or among the three CAD-extension groups. Patients with CAD showed a higher CRP level than those with non-CAD, but there was no difference among the three CAD-extension groups. Patients with 1-VD had a higher serum albumin level than with 2-VD or 3-VD. Patients who showed NYHA functional class $\geq$2 were more prevalent in those with multivessel disease or CAD.

Geometric and functional parameters obtained by echocardiography or cardiac catheterization are shown in Table 3. In all of the studied patients, mean EF was 45.5 ± 1.1% and mean LVMI, LV end-diastolic volume index (LVEDVI) and LV EDWS were 163.5 ± 4.3 g/m^2, 84.4 ± 3.0 ml/m^2 and 35.5 ± 2.0 kdynes/cm^2, respectively.

Table 1. Patient characteristics [1]

	Non-CAD	CAD	*P*-value
N	51	128	
Age (years)	66.5 ± 1.3	68.1 ± 0.8	0.30
Females	9 (18%)	15 (12%)	0.30
BMI (kg/m^2)	22.6 ± 0.4	22.1 ± 0.5	0.50
HT	49 (96%)	120 (94%)	0.51
DM	19 (37%)	72 (56%)	0.02
HLP	15 (29%)	60 (47%)	0.02
AF	2 (4%)	11 (9%)	0.25
OMI	9 (18%)	38 (30%)	0.11
CABG	3 (6%)	20 (16%)	0.23
NYHA class ≥ 2	5 (10%)	44 (34%)	<0.01
HD duration (years)	6.5 ± 1.2	7.7 ± 0.7	0.87
Etiology			
nephrosclerosis	23 (45%)	44 (34%)	0.32
DM	16 (31%)	52 (41%)	0.56
CGN	8 (16%)	26 (20%)	0.61
PCKD	2 (4%)	3 (2%)	0.32
Others	2 (4%)	3 (2%)	0.32
Medication			
ACEI or ARB	23 (45%)	60 (47%)	0.80
β-blocker	29 (57%)	77 (61%)	0.54
Ca blocker	49 (96%)	122 (95%)	0.62
Laboratory			
BNP (pg/ml)	285 ± 30	1237 ± 144	<0.001
Cr (mg/dl)	8.5 ± 0.5	8.7 ± 0.3	0.65
Haemoglobin (g/dl)	10.3 ± 0.1	10.1 ± 0.1	0.39
Albumin (g/dl)	3.70 ± 0.05	3.73 ± 0.04	0.52
CRP (mg/dl)	0.29 ± 0.05	0.52 ± 0.06	0.02

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CGN = chronic glomerulonephritis; Cr = serum creatinine; CRP = C-reactive protein; DM = diabetes mellitus; HD = haemodialysis; HLP = hyperlipidaemia; HT = hypertension; NYHA = New York Heart Association; OMI = old myocardial infarction; PCKD = polycystic kidney disease.
Values are mean ± SEM or number (%).

Cut-off level for detecting CAD

Fifty-one patients who underwent CAG had no significant coronary stenotic lesions. Plasma BNP levels were significantly higher in patients with CAD than in those with non-

Table 2. Patient characteristics [2]

	1-VD	2-VD	3-VD	*P*-value
N	44	33	51	
Age (years)	67.7 ± 1.2	68.1 ± 1.8	68.4 ± 1.2	0.92
Females	2 (5%)	5 (15%)	8 (15%)	0.14
BMI (kg/m^2)	22.0 ± 0.4	21.3 ± 0.5	22.6 ± 1.0	0.28
HT	39 (89%)	33 (100%)	48 (96%)	0.09
DM	20 (46%)	18 (56%)	34 (68%)	0.09
HLP	17 (39%)	16 (49%)	27 (53%)	0.31
AF	4 (9%)	2 (6%)	5 (10%)	0.82
OMI	12 (27%)	9 (27%)	17 (33%)	0.56
CABG	7 (16%)	2 (6%)	11 (22%)	0.12
NYHA class ≥ 2	4 (9%)	13 (44%)	27 (53%)	0.01
HD duration (yrs)	9.9 ± 1.6	7.6 ±1.4	6.2 ± 0.8	0.10
Etiology				
nephrosclerosis	16 (36%)	9 (28%)	19 (37%)	0.32
DM	12 (27%)	15 (46%)	25 (49%)	0.18
CGN	13 (29%)	7 (21%)	6 (12%)	0.08
PCKD	1 (2%)	1 (3%)	1 (2%)	0.32
Others	1 (2%)	1 (3%)	1 (2%)	0.32
Medication				
ACEI or ARB	20 (45%)	14 (44%)	26 (51%)	0.78
β-blocker	28 (63%)	23 (72%)	26 (51%)	0.14
Ca blocker	41 (93%)	32 (96%)	47 (93%)	0.42
Laboratory				
BNP (pg/ml)	496 ± 49	932 ± 119	2073 ± 317	<0.001
Cr (mg/dl)	8.2 ± 0.8	9.1 ± 0.5	8.8 ± 0.4	0.32
Haemoglobin (g/dl)	10.3 ± 0.2	10.2 ± 0.3	10.0 ± 0.2	0.58
Albumin (g/dl)	3.90 ± 0.06	3.65 ± 0.06	3.66 ± 0.06	0.01
CRP (mg/dl)	0.41 ± 0.07	0.49 ± 0.15	0.65 ± 0.11	0.23
Gensini score	33.6 ± 3.4	49.0 ± 4.0	84.7 ± 5.8	<0.001
CAD prognostic index	27.2 ± 1.3	42.6 ± 1.9	68.8 ± 1.7	<0.001

ACEI = angiotensin-converting enzyme inhibitor; AF = atrial fibrillation; ARB = angiotensin receptor blocker; BMI = body mass index; CABG = coronary artery bypass grafting; CAD = coronary artery disease; CGN = chronic glomerulonephritis; CGN = chronic glomerulonephritis; Cr = serum creatinine; CRP = C-reactive protein; DM = diabetes mellitus; HD = haemodialysis; HLP = hyperlipidaemia; HT = hypertension; NYHA = New York Heart Association; OMI = old myocardial infarction; PCKD = polycystic kidney disease.
Values are mean ± SEM or number (%).

Table 3. Echocardiographic and haemodynamic parameters

	Non-CAD	1-VD	2-VD	3-VD	*P*-value
LVEDD (mm)	48.9 ± 1.0	51.6 ± 1.2	52.7 ± 1.3	54.6 ± 1.0	<0.01
PWT (mm)	11.3 ± 0.3	10.3 ± 0.3	11.2 ± 0.3	10.3 ± 0.3	0.01
LAD (mm)	42.3 ± 0.8	43.8 ± 1.0	43.0 ± 1.6	42.7 ± 1.1	0.79
E/A	0.80 ± 0.05	0.98 ± 0.12	0.83 ± 0.07	0.93 ± 0.09	0.44
DCT (msec)	249 ± 9	219 ± 11	226 ± 12	203 ± 10	0.03
LVMI (g/m^2)	152 ± 10	157 ± 6	183 ± 10	167 ± 7	0.02
EF (%)	52.7 ± 1.6	45.8 ± 2.2	44.0 ± 2.6	40.1 ± 1.9	<0.01
LVEDVI (ml/m^2)	73.5 ± 4.1	88.0 ± 5.8	85.0 ± 7.4	90.8 ± 6.5	0.18
LVSP (mmHg)	147 ± 3	145 ± 4	146 ± 4	140 ± 4	0.52
LVEDP (mmHg)	12.4 ± 0.6	13.7 ± 1.0	14.7 ± 1.1	18.4 ± 0.9	<0.01
EDWS (kdynes/cm^2)	23.8 ± 1.6	34.0 ± 5.1	32.5 ± 3.1	48.7 ± 3.8	<0.01

DCT = deceleration time of early diastolic filling; EDWS = end-diastolic wall stress; EF = ejection fraction; E/A = ratio of peak mitral E-wave velocity to peak mitral A-wave velocity; LAD = left atrial dimension; LVEDD = left ventricular end-diastolic dimension; LVEDP = left ventricular end-diastolic pressure; LVEDVI = left ventricular end-diastolic volume index; LVMI = left ventricular mass index; LVSP = left ventricular peak systolic pressure; PWT = posterior wall thickness.
Values are mean ± SEM.

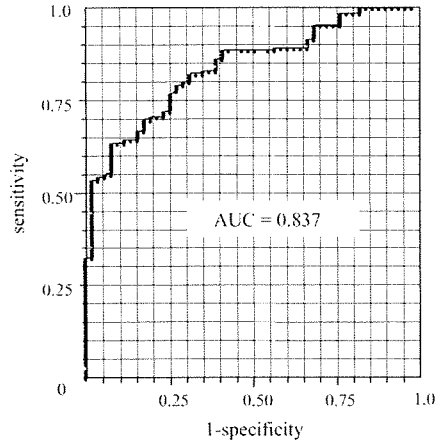

Fig. 1. Receiver operating characteristic (ROC) curve for plasma BNP as a predictor of relevant coronary artery disease. AUC = area under the ROC curve.

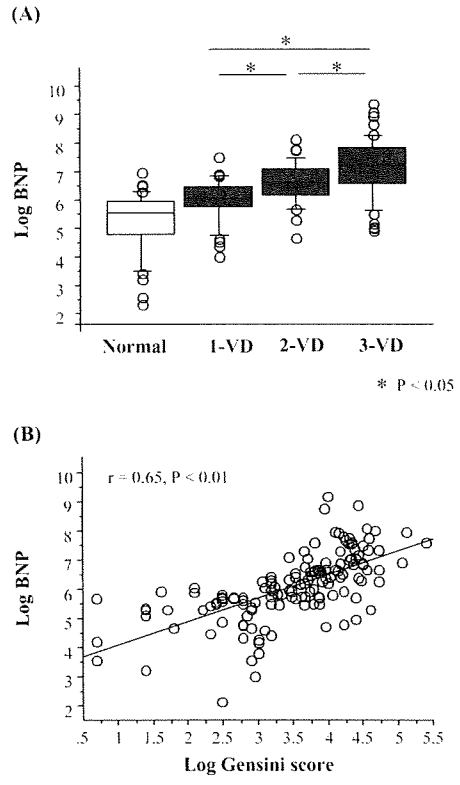

Fig. 2. (**A**) Plasma BNP levels in relation to the number of coronary arteries with >70% diameter stenosis. The box defines the interquartile range with the median indicated by the crossbar. The error bars indicate the 10th and 90th percentiles. (**B**) Correlation between log plasma BNP level and log Gensini score.

CAD (1237 ± 144 and 285 ± 30 pg/ml, respectively; $P < 0.01$). The ROC curve for BNP as an indicator of the presence of CAD is shown in Figure 1. The area under the ROC curve was 0.837 (95% confidence interval 0.778–0.895). The optimal value of BNP as an indicator of CAD was 366 pg/ml, with a sensitivity of 79%, a specificity of 73%, an accuracy of 77%, a positive predictive value of 88% and a negative predictive value of 58%.

CAD extension and plasma BNP levels

Of the 128 patients in the CAD groups, 44, 33 and 51 patients had 1-VD, 2-VD and 3-VD, respectively. As shown in Figure 2, plasma BNP levels increased progressively with the extent of CAD (1-VD, 496 ± 49 pg/ml; 2-VD, 932 ± 119 pg/ml; 3-VD, 2073 ± 317 pg/ml; $P < 0.01$). Furthermore, they correlated well with the Gensini score ($r = 0.65$, $P < 0.01$) or the CAD prognostic index ($r = 0.60$, $P < 0.01$). Thus, plasma BNP levels were directly correlated to the extent of CAD, and the difference between each category was highly significant.

Haemodynamic parameters and plasma BNP levels

In comparisons among the CAD groups, there were no significant differences in LVMI ($P = 0.08$), LV volume ($P = 0.83$) or EF ($P = 0.15$). However, higher LV EDWS and EDP were observed in the 3-VD group ($P < 0.01$), as shown in Table 3. Also, the non-CAD group showed lower LV volume and EDWS and higher EF than CAD group.

As demonstrated in Figure 3, LV EDWS and EF were well correlated with plasma BNP levels ($r = 0.61$ and 0.53, $P < 0.01$, respectively) and LVMI was significantly, but poorly, correlated ($r = 0.27$, $P = 0.009$). Furthermore, a multivariable regression analysis that took into account EDWS demonstrated a significant positive association between the Gensini score, the extent of CAD (the number of diseased vessels), or the CAD prognostic index and plasma BNP levels. In addition, EF was independently associated with plasma BNP level, whereas LVMI and NYHA ≥ 2 were unrelated to plasma BNP once the effects of the EDWS, Gensini score and EF were accounted for (Table 4). The fit (R-square) of the model including these variables was 0.53.

Discussion

CAD is one of the leading causes of morbidity and mortality in chronic HD patients. However, since most patients remain asymptomatic because of deconditioning, limited activity levels and the effects of long-standing DM, the early detection of CAD is difficult. Recently, Charytan *et al.* studied 67 asymptomatic HD patients who volunteered for CAG with a median follow-up of 2.7 years [23]. They showed that 41.7% of the patients had CAD and that the proximal CAD, multivessel disease or the CAD prognostic index >48 was associated with higher mortality. CAG is a definitive diagnostic tool, but it is invasive. It is essential to diagnose the presence and severity of CAD by non-invasive tests as early as possible in HD patients. In the present study, although the area under the ROC curve was <0.85, which makes a BNP test of limited clinical value for detecting CAD, the present result seems to be superior to other reports on patients with no CKD [13] or on those with CKD not requiring dialysis [24]. Also, the diagnostic utility

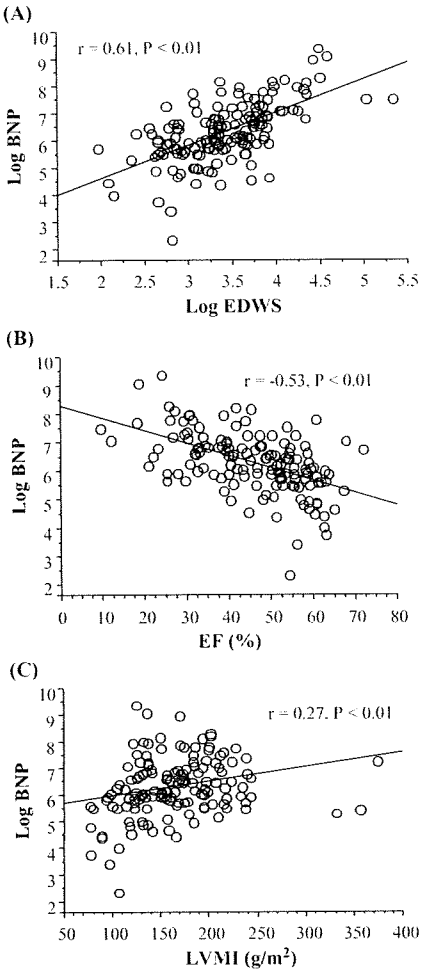

Fig. 3. Correlations between log BNP level and (**A**) log LV end-diastolic wall stress (EDWS), (**B**) LV ejection fraction (EF) and (**C**) LV mass index (LVMI) in all studied patients.

Table 4. Predictors for BNP levels in regression analysis

	Log BNP	
Parameter	β-coefficient	*P*-value
NYHA ≥ 2		NS
LVMI		NS
EF	−0.018	0.009
Log EDWS	0.694	< 0.001
Log Gensini score	0.012	< 0.001

NYHA = New York Heart Association; LVMI = left ventricular mass index; EF = ejection fraction; EDWS = end-diastolic wall stress. Significant univariable predictors were included into the multivariable regression model as continuous and NYHA ≥ 2 as a binary variable.

of other non-invasive tests, including exercise ECG, dobutamine stress echocardiography and scintigraphy for patients with CKD, is reported to be less than that observed in those with no CKD [25]. Thus, the measurement of plasma BNP levels in combination with other non-invasive investigations might help in assessing CAD involvement and aggressive management in this high-risk population.

Several recent studies have suggested that plasma BNP levels may have prognostic potential in chronic HD patients. Zoccali *et al.* demonstrated that BNP was an independent predictor of overall and cardiovascular mortality in HD patients [17]. Cataliotti *et al.* reported that BNP was significantly higher in dialysis patients who died of cardiovascular causes than in survivors [26]. Although LV mass and function have been considered to be important associated factors, ischaemia was not sufficiently considered in these reports. There have been few studies on the association between plasma BNP level and ischaemia itself in chronic HD patients. Although both Osajima *et al.* and Nishikimi *et al.* reported elevated plasma BNP levels in HD patients with CAD [27,28], the diagnostic evaluation of CAD does not seem to be sufficient and the sample number was relatively small. In the present study, we used CAG in all patients for a thorough evaluation of disease severity. The number of diseased coronary arteries, the Gensini score or the CAD prognostic index well correlated with the plasma BNP levels in our study population. Plasma BNP levels may achieve prognostic potential, at least in part, by reflecting the presence and severity of CAD in chronic HD patients. Recently, BNP/NT-proBNP has also been shown to be useful in stable CAD patients with normal renal function. Plasma BNP levels could predict the extent of angiographic coronary artery stenosis and prognosis in patients with stable angina pectoris [13,29] as well as in those with ACS [30]. McClure *et al.* reported that, in patients with coronary ischaemia, removal of coronary stenosis by percutaneous coronary revascularization resulted in decrease of plasma NT-proBNP [31]. Although the pathophysiological mechanism behind the relation between CAD and elevated BNP levels is not well defined, Goetze *et al.* reported that tissue hypoxia alone could trigger release of BNP in the absence of LV dysfunction [10]. We recently demonstrated that LV EDWS is a crucial haemodynamic determinant of plasma BNP levels in patients with chronic heart failure [7]. Therefore, we measured LV EDWS and tried to clarify the independent role of chronic ischaemia on plasma BNP from haemodynamic load in the setting of CAD in HD patients. As a result, LV EDWS was associated with plasma BNP levels, but to a lesser extent than in patients with heart failure [7,32]. The extent of CAD (including the Gensini score or the CAD prognostic index) was correlated with plasma BNP levels independent of the haemodynamic load according to a multivariable analysis. Chronic ischaemia itself might contribute to the elevated BNP levels in the present setting.

Several limitations should be considered in interpreting our results. First, the study population was relatively small, especially in the non-CAD group. Any negative findings could thus be caused by a low statistical power. Second, only plasma BNP levels were considered in our study. Recently, the measurement of NT-proBNP was increasingly used clinically because of its longer half-life and larger size. NT-proBNP might be more dependent on renal clearance than BNP [33]. However, most studies have demonstrated that both are equally useful, even in CKD and HD patients [34,35]. Third, echocardiography was typically performed

the day before cardiac catheterization. This time lag could have influenced the results. Last, in the present study, a cohort of HD patients consisted of those who had been referred for CAG due to symptoms or objective evidence of ischaemia, and asymptomatic patients with lack of objective evidence of ischaemia were not included. Therefore, the applicability of our results to the screening for CAD in all HD patients might be limited.

The present study clearly showed that plasma BNP levels were closely correlated with disease severity as assessed by the number of stenotic coronary arteries, the Gensini score and CAD prognostic index in chronic HD patients with CAD. In addition, they had significantly higher plasma BNP levels than those with non-CAD. We also analyzed EDWS and showed that chronic ischaemia itself might contribute to the increased BNP levels in addition to the EDWS in this setting. Therefore, our data suggest that plasma BNP levels may be a useful marker in the diagnosis and follow-up of stable CAD in patients with chronic HD by reflecting both the haemodynamic load and the presence and severity of ischaemia.

Acknowledgements. This study was supported by a research grant from the ONO Medical Research Foundation, a Japan Heart Foundation/Novartis Grant for Research Award on Molecular and Cellular Cardiology and a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (18590774).

Conflict of interest Statement. None declared.

References

1. Villar E, Remontet L, Labeeuw M *et al.* Effect of age, gender, and diabetes on excess death in end-stage renal failure. *J Am Soc Nephrol* 2007; 18: 2125–2134
2. U.S. Renal Data System.: Atlas of End-Stage Renal Disease in the United States. *USRDS 2004 Annual Data Report.* National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, 2004
3. Collins AJ. Cardiovascular mortality in end-stage renal disease. *Am J Med Sci* 2003; 325: 163–167
4. Lindner A, Charre B, Sherrard DJ *et al.* Accelerated atherosclerosis in prolonged maintenance hemodialysis. *N Engl J Med* 1974; 290: 697–701
5. Rostand SG, Gretes JC, Kirk KA *et al.* Ischemic heart disease in patients with uremia undergoing maintenance hemodialysis. *Kidney Int* 1979; 16: 600–611
6. Nakagawa O, Ogawa Y, Itoh H *et al.* Rapid transcriptional activation and early mRNA turnover of brain natriuretic peptide in cardiocytehypertrophy; evidence for brain natriuretic peptide as an "emergency" cardiac hormone against ventricular overload. *J Clin Invest* 1995; 96: 1280–1287
7. Iwanaga Y, Nishi I, Furuichi S *et al.* B-type natriuretic peptide strongly reflects diastolic wall stress in patients with chronic heart failure: comparison between systolic and diastolic heart failure. *J Am Coll Cardiol* 2006; 47: 742–748
8. Morrow DA, de Lemos JA, Blazing MA *et al.* Prognostic value of serial B-type natriuretic peptide testing during follow-up of patients with unstable coronary artery disease. *JAMA* 2005; 294: 2866–2871
9. Konstam MA. Natriuretic peptides and cardiovascular events: more than a stretch. *JAMA* 2007; 297: 212–214
10. Goetze JP, Christoffersen C, Perko M. Increased cardiac BNP expression associated with myocardial ischemia. *FASEB J* 2003; 17: 1105–1107
11. Bibbins-Domingo K, Ansari M, Sciller NB. B-type natriuretic peptide and ischemia in patients with stable coronary artery disease: data from the Heart and Soul study. *Circulation* 2003; 108: 2987–2992
12. Bibbins-Domingo K, Gupta R, Na B *et al.* N-Terminal fragment of the prohormone brain-type natriuretic peptide (NT-proBNP), cardiovascular events, and mortality in patients with stable coronary heart disease. *JAMA* 2007; 297: 169–176
13. Weber M, Dill T, Arnold R *et al.* N-Terminal B-type natriuretic peptide predicts extent of coronary artery disease and ischemia in patients with stable angina pectoris. *Am Heart J* 2004; 148: 612–620
14. Ishizaka Y, Yamamoto Y, Fukunaga T *et al.* Plasma concentration of human brain natriuretic peptide in patients on hemodialysis. *Am J Kidney Dis* 1994; 24: 461–472
15. McCullough PA, Sandberg KR. Sorting out the evidence on natriuretic peptides. *Rev Cardiovasc Med* 2003; 4(Suppl 4): S13–S19
16. Naganuma T, Sugimura K, Wada S *et al.* The prognostic role of brain natriuretic peptides in hemodialysis patients. *Am J Nephrol* 2002; 22: 437–444
17. Zoccali C, Mallamaci F, Benedetto FA *et al.* Cardiac natriuretic peptides are related to left ventricular mass and function and predict mortality in dialysis patients. *J Am Soc Nephrol* 2001; 12:1508–1515
18. Wang AY, Lam CW, Yu CM *et al.* N-Terminal pro-brain natriuretic peptide: an independent risk predictor of cardiovascular congestion, mortality, and adverse cardiovascular outcomes in chronic peritoneal dialysis patients. *J Am Soc Nephrol* 2007; 18: 321–330
19. Gensini GG. A more meaningful scoring system for determining the severity of coronary heart disease. *Am J Cardiol* 1983; 51: 606
20. Bart BA, Shaw LK, McCants CB Jr *et al.* Clinical determinants of mortality in patients with angiographically diagnosed ischemic or nonischemic cardiomyopathy. *J Am Coll Cardiol* 1997; 30: 1002–1008
21. Felker GM, Shaw LK, O'Connor CM. A standardized definition of ischemic cardiomyopathy for use in clinical research. *J Am Coll Cardiol* 2002; 39: 210–218
22. Schiller NB, Shah PM, Crawford M *et al.* Recommendations for quantitation of the left ventricle by two-dimensional echocardiography. *J Am Soc Echocardiogr* 1989; 2: 358–367
23. Charytan D, Kuntz RE, Mauri L *et al.* Distribution of coronary artery disease and relation to mortality in asymptomatic hemodialysis patients. *Am J Kidney Dis* 2007; 49: 409–416
24. DeFilippi CR, Fink JC, Nass CM *et al.* N-terminal pro-B-type natriuretic peptide for predicting coronary disease and left ventricular hypertrophy in asymptomatic CKD not requiring dialysis. *Am J Kidney Dis* 2005; 46: 35–44
25. Murphy SW. Management of heart failure and coronary artery disease in patients with chronic kidney disease. *Semin Dial* 2003; 16: 165–172
26. Cataliotti A, Malatino LS, Jougasaki M *et al.* Circulating natriuretic peptide concentrations in patients with end-stage renal disease: role of brain natriuretic peptide as a biomarker of ventricular remodeling. *Mayo Clin Proc* 2001; 76: 1111–1119
27. Osajima A, Okazaki M, Tamura M *et al.* Comparison of plasma levels of mature adrenomedullin and natriuretic peptide as markers of cardiac function in hemodialysis patients with coronary artery disease. *Nephron* 2002; 92: 832–839
28. Nishikimi T, Futoo Y, Tamano K *et al.* Plasma brain natriuretic peptide levels in chronic hemodialysis patients: influence of coronary artery disease. *Am J Kidney Dis* 2001; 37: 1201–1208
29. Kragelund C, Gronning B, Kober L *et al.* N-terminal pro-B-type natriuretic peptide and long-term mortality in stable coronary heart disease. *N Engl J Med* 2005; 352: 666–675
30. Choi EY, Kwon HM, Yoon YW *et al.* Assessment of extent of myocardial ischemia in patients with non-ST elevation acute coronary syndrome using serum B-type natriuretic peptide level. *Yonsei Med J* 2004; 45: 255–262
31. McClure SJ, Gall S, Schechter CB *et al.* Percutaneous coronary revascularization reduces plasma N-terminal pro-B-type natriuretic peptide

concentration in stable coronary artery disease. *J Am Coll Cardiol* 2007; 49: 2394–2397
32. Iwanaga Y, Kihara Y, Niizuma S *et al.* BNP in overweight and obese patients with heart failure: an analysis based on the BNP-LV diastolic wall stress relationship. *J Card Fail* 2007; 13: 663–667
33. McCullough PA, Sandberg KR. B-type natriuretic peptide and renal disease. *Heart Fail Rev* 2003; 8: 355–358
34. Wahl HG, Graf S, Renz H *et al.* Elimination of the cardiac natriuretic peptides B-type natriuretic peptide (BNP) and N-terminal proBNP by hemodialysis. *Clin Chem* 2004; 50: 1071–1074
35. Clerico A, Fontana M, Zyw L *et al.* Comparison of the diagnostic accuracy of brain natriuretic peptide (BNP) and the N-terminal part of the propeptide of BNP immunoassays in chronic and acute heart failure: a systematic review. *Clin Chem* 2007; 53: 813–822

Received for publication: 23.5.08
Accepted in revised form: 7.8.08

Obesity Research & Clinical Practice (2009) 3, 65—74

ELSEVIER

http://www.iotf.org/oonet/aoaso.htm

ORIGINAL ARTICLE

Resistin gene variations are associated with the metabolic syndrome in Japanese men

Yoshihiro Miyamoto[a,*], Hiroko Morisaki[b], Yoshihiro Kokubo[c], Itaru Yamanaka[b], Hitonobu Tomoike[d], Akira Okayama[c], Yasunao Yoshimasa[a], Takayuki Morisaki[b]

[a] *Department of Atherosclerosis and Diabetes, National Cardiovascular Center, 5-7-1 Fujishirodai, Suita, Osaka 565-8565, Japan*
[b] *Department of Bioscience, National Cardiovascular Center Research Institute, Osaka, Japan*
[c] *Department of Preventive Cardiology, National Cardiovascular Center, Osaka, Japan*
[d] *National Cardiovascular Center Hospital, Osaka, Japan*

Received 12 March 2008; received in revised form 16 September 2008; accepted 18 November 2008

KEYWORDS
Resistin;
Metabolic syndrome;
Genetic epidemiology;
Risk factors

Summary
Objectives: Metabolic syndrome is defined as a cluster of risk factors for cardiovascular disease and is intimately related to insulin resistance. Resistin, a hormone secreted by adipocytes, may play an important role in communication between adiposity and insulin resistance. We investigated whether variations in the resistin gene associated with metabolic syndrome in a Japanese population.
Method: We analyzed five SNPs, two of which were located in the promoter region (−420C > G, −358G > A), two in intron 2 (+157C > T, +299G > A), and one in the 3′-untranslated region (3′UTR) (+1263G > C) across the resistin gene in 2968 residents from an urban Japanese cohort. The associations of SNPs and haplotypes with metabolic syndrome were analyzed.
Results: The GAC and CGC haplotypes (comprising −420C > G, −358G > A, and +157C > T) had opposite influences on metabolic syndrome susceptibility in men; the former was associated with an increased risk and the latter with a decreased risk. We also found that the −420G allele was significantly associated with an increased risk of metabolic syndrome and significantly correlated with high diastolic blood pressure, high HOMA-IR values, high serum triglyceride levels, low HDL-cholesterol levels and high serum levels of adiponectin.

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6872 7486.
E-mail address: miyamoty@hsp.ncvc.go.jp (Y. Miyamoto).

1871-403X/$ – see front matter.
doi:10.1016/j.orcp.2008.11.003

Conclusion: We identified a risk-conferring SNP and haplotype of the resistin gene for metabolic syndrome in a Japanese population. Our data suggested that resistin gene is a susceptibility gene for metabolic syndrome in Japanese men.

Introduction

Metabolic syndrome is defined as a cluster of metabolic abnormalities, including obesity, glucose intolerance, dyslipidemia, and hypertension [1,2]. Metabolic syndrome promotes atherosclerosis, leading to cardiovascular disease, and increases the risk of type 2 diabetes. Because type 2 diabetes is a well-known risk factor for cardiovascular disease, metabolic syndrome has long been recognized as an important underlying cause of cardiovascular problems [3].

Epidemiologic studies indicate that metabolic syndrome has become more prevalent in both Western and Asian countries as lifestyle choices such as a high-calorie diet and sedentary behavior have become more common. These studies indicate that environmental factors influence the prevalence of metabolic syndrome [4]. In addition, a genetic predisposition for metabolic syndrome has also been demonstrated [6–16].

Recent evidence indicates that adipocytes secrete several molecules that effect glucose metabolism and insulin sensitivity, such as fatty acids, adiponectin, leptin, and interleukin-6, while visceral obesity impairs or modulates the function of these hormones and thus leads to metabolic syndrome [5]. Resistin is a hormone that is secreted from adipocytes and down-regulated by thiazolidinediones [6]. These drugs are peroxisome proliferator-activated receptor-gamma (PPARγ) agonists that improve insulin resistance by activating genes containing PPARγ responsive elements, including genes involved in regulating glucose metabolism and insulin sensitivity [7]. Therefore, it has been proposed that resistin may crucially link adiposity to insulin resistance. Steppan et al. have shown that administration of recombinant resistin induces hyperglycemia and insulin resistance, while infusion of anti-resistin antibodies ameliorates these changes [8]. Subsequent studies have indicated that mice with the null allele of the resistin gene are protected against hyperglycemia when fed a high-fat diet, because resistin deficiency leads to decreased hepatic glucose production without affecting whole-body glucose disposal [9]. Thus, a significant role of resistin in glucose metabolism is well documented in rodents. However, the role of resistin in human glucose metabolism and related diseases remains controversial [10–12].

Some clues about the influence of resistin on glucose metabolism in humans have been obtained from genetic studies in certain populations. Engert et al. and Conneely et al. identified resistin gene variants that were associated with obesity and type 2 diabetes in humans [13,14]. However, these associations have been inconsistent, probably due to differences in sample size, ethnicity, and disease status [15–19]. In light of the possible involvement of resistin in insulin resistance and the regulation of resistin gene expression by thiazolidinediones, we investigated whether variations of the resistin *gene* were associated with metabolic syndrome in an urban Japanese population.

Methods

Subjects and definition of metabolic syndrome

We recruited and obtained written informed consent for 3655 participants from Suita city (Osaka Prefecture, Japan) during routine physical checks from April 2002 to February 2004. The study design was approved by the institutional research board and ethics committee of the National Cardiovascular Center and by the Committee on Genetic Analysis and Gene Therapy of the National Cardiovascular Center. Among 3655 participants, 2968 persons were included in the analysis because we could collect blood after a 12-h fast and because all five single nucleotide polymorphisms (SNPs) of the resistin gene were successfully genotyped in these subjects. According to the Japanese consensus definition, metabolic syndrome is defined as central obesity (waist circumference ≥85 cm for men and ≥90 cm for women) plus any two of the following three factors: dyslipidemia (triglycerides ≥1.69 mmol/l (150 mg/dl) and/or high-density lipoprotein (HDL) cholesterol ≤1.03 mmol/l (40 mg/dl) or lipid-lowering therapy), hypertension (systolic BP ≥130 and/or diastolic BP ≥85 mmHg, or antihypertensive therapy), and fasting plasma glucose ≥6.11 mmol/l

Table 1 Comparison of clinical parameters among metabolic syndrome, intermediate and control groups in an urban Japanese cohort ($n=2968$).

	Control ($n=765$)	Intermediate ($n=1779$)	MS ($n=424$)	P^*
Men (n, %)	197, 25.8	833, 46.8	324, 76.4	<0.001
Age (year)	59.5 ± 11.5	67.9 ± 10.0	67.5 ± 9.6	<0.001
Smoking (n, %)	114, 14.9	246, 13.8	98, 23.1	<0.001
Drinking (n, %)	286, 37.4	796, 44.7	235, 55.4	<0.001
BMI (kg/m^2)†	20.8 ± 2.3	22.9 ± 2.9	25.9 ± 2.7	<0.001
Waist (cm)†	77.3 ± 6.3	85.0 ± 8.0	93.0 ± 6.0	<0.001
SBP (mmHg)†	112.1 ± 9.9	135.3 ± 18.2	141.0 ± 16.1	<0.001
DBP (mmHg)†	71.0 ± 7.5	79.3 ± 9.4	83.7 ± 9.7	<0.001
FBG (mmol/l)†	5.02 ± 0.42	5.52 ± 1.04	6.59 ± 1.76	<0.001
HbA_{1c} (%)†	5.2 ± 0.3	5.5 ± 0.7	6.1 ± 1.1	<0.001
HOMA-IR†	0.89 ± 0.55	1.38 ± 1.05 (1)	2.77 ± 2.57	<0.001
T-Cho (mmol/l)	5.35 ± 0.83	5.40 ± 0.82	5.35 ± 0.92	0.786
TG (mmol/l)†	0.83 ± 0.31	1.19 ± 0.68	1.89 ± 0.99	<0.001
HDLc (mmol/l)†	1.75 ± 0.39	1.55 ± 0.39	1.28 ± 0.33	<0.001
LDLc (mmol/l)	3.22 ± 0.76	3.30 ± 0.76	3.21 ± 0.81	0.816
Leptin (ng/ml)†	10.1 ± 4.5	12.1 ± 6.8 (1)	13.7 ± 7.8	<0.001
Adiponectin (ng/ml)†	10.4 ± 5.3 (6)	9.0 ± 5.3 (9)	5.9 ± 3.9	<0.001

MS, metabolic syndrome; SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; T-Cho, total cholesterol; HDLc, HDL cholesterol; LDLc, LDL cholesterol. Data are shown as the mean ± S.D. The laboratory data reported in milligram per deciliter can be converted to SI units as follows: total cholesterol, HDL cholesterol and LDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l. Numbers of missing data for each parameter are indicated in parenthesis next to the mean ± S.D.

* P-values for comparison between metabolic syndrome and control groups.

† P-values for the trend among the three groups of the parameters were less than 0.05.

(110 mg/dl) or previously diagnosed type 2 diabetes [20]. Subjects that did not meet the metabolic syndrome criteria were defined as intermediates if they met one or more of the above criteria or as controls if they had none of these criteria. Among 2968 persons, we identified 424 metabolic syndrome subjects, 1779 intermediate subjects, and 765 controls (Table 1).

As for evaluating the relation between the resistin genotype and plasma concentration of it, we recruited and obtained written informed consent for 169 volunteers from Yahaba town (Iwate Prefecture, Japan).

Clinical parameters

Blood pressure was measured after at least 10 min of rest in the sitting position. The mean value of 2 SBP or DBP measurements obtained by a physician using a mercury sphygmomanometer (recorded >3 min apart) was used for analysis. Subjects were classified as current smokers or drinkers if they still smoked or drank. After 12 h of fasting, blood samples were collected into tubes containing EDTA. Total cholesterol and HDL cholesterol levels were measured with an autoanalyzer (Toshiba TBA-80) 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. Plasma concentrations of resistin were measured by radioimmunoassay (SRL, Inc., Tokyo, Japan).

Screening and identification of SNPs in the resistin gene

DNA samples were isolated from peripheral blood leukocytes of participants using an NA-3000 (Kurabo Industries Ltd., Osaka, Japan). Five primers sets were designed to amplify the promoter region, exons, and intron/exon boundaries of the resistin gene. The initial SNP screening was performed using 24 randomly chosen DNA samples. Screening for genetic variants was performed by the denaturing HPLC method, in which the PCR products were analyzed using the WAVE DNA Fragment Analysis and WAVEMAKER software 4.0 (Transgenomic, Inc., Omaha, NE, USA) according to the manufacturer's protocol. All detected variations were further confirmed by direct sequencing using an ABI 3700 (Applied Biosystems, Foster City, CA, USA). SNPs were genotyped by TaqMan PCR (ABI PRISM 7900HT, Applied Biosystems). The validity of these detection systems was verified prior to the large-scale study, using 24 samples that were genotyped at the initial screening. All SNPs analyzed in this study

were verified by two different genotyping methods.

Estimation of haplotype frequencies and evaluation of linkage disequilibrium of the resistin gene

Haplotypes and the linkage disequilibrium coefficient (D' and r^2-values) were computed using Haploview software, version 3.32 (http://www.broad.mit.edu/personal/jcbarret/haploview).

Statistical analysis

We analyzed an urban Japanese cohort that was divided into the following three groups: metabolic syndrome subjects, intermediates, and controls. Clinical parameters were compared between the metabolic syndrome and control groups by a Dunnett test and the trend analysis for clinical parameters among the three groups was performed by the Tukey–Kramer HSD test. Data on fasting blood glucose, HOMA-IR, triglyceride, leptin, and adiponectin levels were transformed to natural logarithm values before analysis. The following numbers are missing from the data: a HOMA-IR value, an LDL-cholesterol value, a leptin level, and 15 adiponectin levels.

We analyzed the association between the risk haplotype and metabolic syndrome by the χ^2-test using Haploview software. The genotypic relative risk comparing the metabolic syndrome group with the control group was assessed by calculating the odds ratio (OR) and the 95% confidence interval (C.I.), using logistic regression analysis after adjusting for age and sex. Clinical variables between subjects with and without the risk allele were compared by a logistic regression analysis with adjustments for age and sex.

All P-values were two-tailed, and P-values below 0.05 were considered statistically significant. All statistical analyses without association studies of haplotypes were performed using JMP software, version 6.0 (SAS Institute, Inc., Cary, NC).

Results

Clinical features of metabolic syndrome

Table 1 shows the clinical characteristics of the control subjects and metabolic syndrome subjects. The metabolic syndrome group was predominantly men (men/women ratio: 324/100) and older than control subjects (67.5 ± 9.6 vs. 59.5 ± 11.5 years). The body mass index, waist circumference, systolic and diastolic blood pressure, fasting blood glucose, hemoglobin A_{1C}, and triglyceride levels of the metabolic syndrome group were significantly higher and HDL-cholesterol was significantly lower than the control groups, reflecting the criteria used to define this syndrome. Total cholesterol and LDL-cholesterol were not significantly different between the metabolic syndrome and control groups. The serum leptin and adiponectin levels of subjects with metabolic syndrome were significantly higher and lower than those of the control group, respectively, suggesting an abnormal body fat distribution in the former group.

Identification of resistin gene polymorphisms

Twenty-four individuals were examined for resistin gene polymorphisms, including all four exons (Genbank accession number: AF352730, nt 2316–4913), using the WAVE system. A total of 10 SNPs were found, and the five SNPs with the highest frequencies were selected (Table 2). All five SNPs were in Hardy–Weinberg equilibrium, and were reported in the IMS-JST SNPs database (http://snp.ims.u-tokyo.ac.jp/index.html) or in the NCBI db SNP database (http://www.ncbi.nlm.nih.gov/SNP/). Two of the five SNPs were located in the promoter region (−420C > G, −358G > A), two in intron 2 (+157C > T, +299G > A) and one in the 3′-untranslated region (3′UTR) (+1263G > C).

Evaluation of linkage disequilibrium

Using these five SNPs as tags to define haplotypes, we evaluated the pattern of linkage disequilibrium in the 2968 subjects. As shown in Fig. 1, there was one linkage disequilibrium block in this population, and SNP-1 (−420C > G), SNP-2 (−358G > A), and SNP-3 (+157C > T) were in strong linkage disequilibrium. Thus, these three SNPs (SNP-1, -2, and -3) were used to define haplotypes.

Association of resistin gene variations with metabolic syndrome

An analysis of the association between variations in the resistin gene and metabolic syndrome showed that a haplotype comprising SNP-1, -2, and -3 conferred significant susceptibility to metabolic syndrome in men (Table 3). The GAC haplotype was associated with a significantly increased risk of metabolic syndrome among men but not women (metabolic syndrome 23.1%, control

Table 2 Characteristics of the resistin gene polymorphisms.

SNP	Position[a] genome	JSNP ID[b]	dbSNP ID[c]	Major/minor	Location	Frequency of minor allele[d]
1	−420		rs1862513	C/G	5′flanking	0.340
2	−358	096816	rs3219175	G/A	5′flanking	0.206
3	+157	096817	rs3219177	C/T	Intron2	0.064
4	+299	096818	rs3745367	G/A	Intron2	0.383
5	+1263	096820	rs3745369	G/C	3′UTR	0.282

[a] Numbers indicate locations relative to the A of the ATG translation initiation codon.
[b] JSNP is a repository of Japanese Single Nucleotide Polymorphism (SNP) data (http://snp.ims.u-tokyo.ac.jp/index.html).
[c] dbSNP is a database of Single Nucleotide Polymorphisms built by National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/).
[d] Based on the result of screening all samples ($n = 2968$).

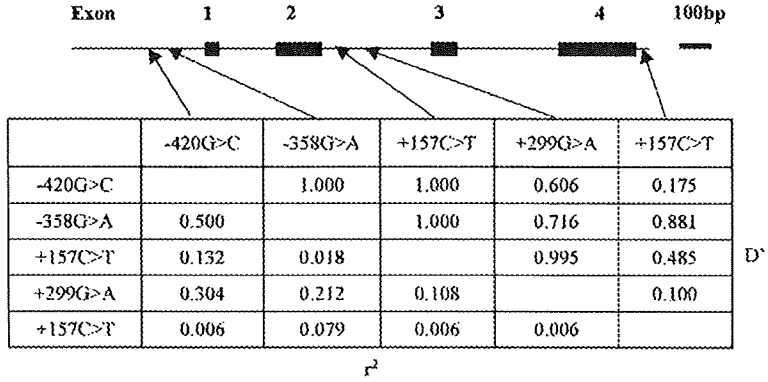

Figure 1 Using five SNPs (−420C > G, −358G > A, +157C > T, +299G > A, +1263G > C) as tags to define haplotypes, we calculated the pair-wise r^2 and D' for each SNP pair and evaluated the linkage disequilibrium pattern for the resistin gene in 2968 subjects.

Table 3 Frequency of haplotypes in a linkage disequilibrium block between SNP-1 and SNP-3 of the resistin gene and the association with metabolic syndrome.

1 2 3	All	MS	Control	χ^2	*P*-value	OR	95%C.I.
Men + women							
CGC	0.660	0.629	0.675	5.275	**0.022**	**0.81**	**0.68–0.97**
GAC	0.206	0.228	0.190	4.707	**0.030**	**1.25**	**1.02–1.54**
GGC	0.070	0.080	0.066	1.661	0.198	1.23	0.90–1.70
GGT	0.064	0.064	0.069	0.214	0.644	0.92	0.66–1.30
Men							
CGC	0.661	0.636	0.703	4.946	**0.026**	**0.73**	**0.56–0.96**
GAC	0.209	0.231	0.165	6.618	**0.010**	**1.52**	**1.10–2.11**
GGT	0.066	0.063	0.063	0.000	0.991	1.00	0.60–1.67
GGC	0.065	0.069	0.069	0.003	0.955	1.01	0.62–1.66
Women							
CGC	0.659	0.605	0.665	2.759	0.097	0.77	0.57–1.05
GAC	0.204	0.215	0.199	0.273	0.602	1.10	0.76–1.59
GGC	0.075	0.115	0.065	6.279	**0.012**	**1.86**	**1.14–3.06**
GGT	0.063	0.065	0.070	0.077	0.781	0.92	0.50–1.68

MS, metabolic syndrome; 95%C.I., 95% confidential index. Haplotypes significantly associated with metabolic syndrome are in bold.

Table 4 Distribution of resistin SNP genotypes in metabolic syndrome subjects.

	Men + women		Men		Women	
	MS (n = 424)	Control (n = 765)	MS (n = 324)	Control (n = 197)	MS (n = 100)	Control (n = 568)
−420C > G						
CC	169(39.9)	347(45.4)	130(40.1)	100(50.8)	39(39.0)	247(43.5)
CG	195(46.0)	339(44.3)	152(46.9)	77(39.1)	43(43.0)	262(46.1)
GG	60(14.1)	79(10.3)	42(13.0)	20(10.2)	18(18.0)	59(10.4)
OR (95%C.I.)	1.5(1.1–2.0)		1.6(1.1–2.3)		1.2(0.7–1.9)	
P	0.004		0.008		0.499	
−358 G > A						
GG	253(59.7)	496(64.8)	192(59.3)	136(69.0)	61(61.0)	360(63.4)
GA	149(35.1)	247(32.3)	114(35.2)	57(28.9)	35(35.0)	190(33.5)
AA	22(5.2)	22(2.9)	18(5.6)	4(2.0)	4(4.0)	18(3.2)
OR (95%C.I.)	1.3(1.0–1.8)		1.5(1.1–2.3)		1.0(0.6–1.7)	
P	0.047		0.024		0.873	
+157C > T						
CC	372(87.7)	664(86.8)	285(88.0)	172(87.3)	87(87.0)	492(86.6)
CT	50(11.8)	97(12.7)	37(11.4)	25(12.7)	13(13.0)	72(12.7)
TT	2(0.5)	4(0.5)	2(0.6)	0(0.0)	0(0.0)	4(0.7)
OR (95%C.I.)	1.1(0.7–1.6)		1.0(0.6–1.7)		1.1(0.6–2.1)	
P	0.760		1.000		0.730	
+299G > A						
GG	143(33.7)	295(38.6)	107(33.0)	79(40.1)	36(36.0)	216(38.0)
GA	206(48.6)	380(49.7)	157(48.5)	95(48.2)	49(49.0)	285(50.2)
AA	75(17.7)	90(11.8)	60(18.5)	23(11.7)	15(15.0)	67(11.8)
OR (95%C.I.)	1.4(1.0–1.8)		1.4(1.0–2.0)		1.2(0.8–2.1)	
P	0.043		0.071		0.308	
+1263G > C						
GG	211(49.8)	384(50.2)	166(51.2)	99(50.3)	45(45.0)	285(50.2)
GC	186(43.9)	319(41.7)	140(43.2)	79(40.1)	46(46.0)	240(42.3)
CC	27(6.4)	62(8.1)	18(5.6)	19(9.6)	9(9.0)	43(7.6)
OR (95%C.I.)	1.1(0.8–1.4)		1.0(0.7–1.4)		1.1(0.7–1.7)	
P	0.538		0.963		0.699	

MS, metabolic syndrome. Odds ratio and 95%C.I. are for the dominant model of the minor allele. P-values were calculated using a logistic regression analysis after adjusting for age and sex or for age only.

16.5%; OR = 1.52, 95%C.I., 1.10–2.11; $P = 0.010$), while the CGC haplotype was associated with a decreased risk of metabolic syndrome, also only in men (metabolic syndrome 63.6%, control 70.3%; OR = 0.73, 95%C.I., 0.56–0.96; $P = 0.026$). After permutation tests ($n = 1000$), the association between the GAC haplotype and metabolic syndrome remained significant ($P = 0.046$) while that between CGC and metabolic syndrome did not ($P = 0.094$).

The −358A allele is a representative SNP of the GAC haplotype of SNP-1, -2, and -3 and contributes to an increased risk of metabolic syndrome. Assuming a dominant model, the −358A polymorphism was associated with an increased risk of developing metabolic syndrome in Japanese individuals (OR = 1.3, 95%C.I., 1.0–1.8; $P = 0.047$). However, as shown in Table 4, the −420G allele had a higher odds ratio in metabolic syndrome subjects than controls after adjusting for sex and age (OR = 1.5, 95%C.I., 1.1–2.0; $P = 0.004$). Moreover, the +299G > A SNP in intron 2 was also dominantly associated with metabolic syndrome in this Japanese cohort. As the +299G > A SNP was not in linkage disequilibrium with either SNP-420C > G or SNP-358G > A, the +299A allele may be another putative marker SNP for metabolic syndrome that is unrelated to the −420C > G SNP. However, there was no significant association with the +299A allele in both men and women subgroups. Moreover, the −420C > G SNP and −358G > A SNP were significantly associated with metabolic syndrome only in men.

Association of the −420C > G SNP with clinical parameters in urban Japanese men and women

Table 5 shows the association of these SNPs with various clinical parameters using an analysis of covariance, after adjusting for age. Diastolic blood pressures in men with the −420CG + GG genotype were significantly higher than those in men with the −420CC genotype. Men with the −420CG + GG genotype also had higher serum triglyceride levels and lower serum HDL cholesterol levels than those with the −420CC genotype. Insulin resistance by the homeostasis model of assessment (HOMA-IR) value was significantly higher in those with the −420CC + CG genotype than in those with the −420CC genotype (1.66 ± 0.02 vs. 1.50 ± 0.06; $P = 0.043$). Moreover, serum adiponectin levels in men with the −420CG + GG genotype were significantly lower than levels in men with the −420CC genotype.

Table 5 Comparison of clinical parameters in urban Japanese men and women ($n = 2968$) according to resistin −420C > G genotype.

	Men ($n = 1354$)			Women ($n = 1614$)		
	CC ($n = 591$)	CG + GG ($n = 763$)	P	CC ($n = 694$)	CG + GG ($n = 920$)	P
Age	68.3 ± 10.6	67.0 ± 10.7	0.026	64.5 ± 11.1	63.8 ± 10.9	0.165
BMI	23.0 ± 0.1	23.3 ± 0.1	0.081	22.3 ± 0.1	22.4 ± 0.1	0.340
Waist (cm)	85.3 ± 0.3	85.8 ± 0.3	0.290	83.3 ± 0.4	83.2 ± 0.3	0.877
SBP (mmHg)	131.9 ± 0.8	133.2 ± 0.7	0.215	130.6 ± 0.8	129.7 ± 0.6	0.364
DBP (mmHg)	78.3 ± 0.4	79.5 ± 0.4	0.028	76.5 ± 0.4	76.9 ± 0.3	0.357
FBG (mmol/l)	5.72 ± 0.06	5.78 ± 0.05	0.316	5.37 ± 0.03	5.41 ± 0.04	0.524
HbA_{1c} (%)	5.60 ± 0.04	5.62 ± 0.03	0.684	5.40 ± 0.02	5.67 ± 0.02	0.099
HOMA-IR	1.50 ± 0.06	1.66 ± 0.02	0.043	1.34 ± 0.04	1.35 ± 0.04	0.527
T-Cho (mmol/l)	5.08 ± 0.03	5.14 ± 0.03	0.125	5.63 ± 0.03	5.58 ± 0.03	0.153
TG (mmol/l)	1.25 ± 0.03	1.39 ± 0.03	0.002	1.08 ± 0.02	1.08 ± 0.02	0.985
HDLc (mmol/l)	1.45 ± 0.02	1.39 ± 0.01	0.002	1.68 ± 0.02	1.67 ± 0.01	0.894
LDLc (mmol/l)	3.05 ± 0.03	3.12 ± 0.03	0.091	3.46 ± 0.03	3.41 ± 0.03	0.160
Leptin (ng/ml)	9.2 ± 0.2	9.4 ± 0.2	0.827	14.1 ± 0.3	13.9 ± 0.2	0.578
Adiponectin (mg/ml)	7.8 ± 0.2	7.2 ± 0.2	0.009	10.7 ± 0.2	10.4 ± 0.2	0.310

SBP, systolic blood pressure; DBP, diastolic blood pressure; FBG, fasting blood glucose; TG, triglycerides; T-Cho, total cholesterol; HDLc, HDL cholesterol; LDLc, LDL cholesterol. Data except for age are shown as the adjusted means ± S.E. These values were obtained after adjusting for age by the least squares method. Age is shown as the mean ± S.D. The laboratory data reported in milligram per deciliter were converted to SI units as follows: total cholesterol, HDL cholesterol and LDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l. Data on FBG, HOMA-IR, TG, leptin, and adiponectin were transformed to natural logarithm values before analysis. Numbers of missing data in all samples were one HOMA-IR value, one LDLc level, one leptin level and 15 adiponectin levels.

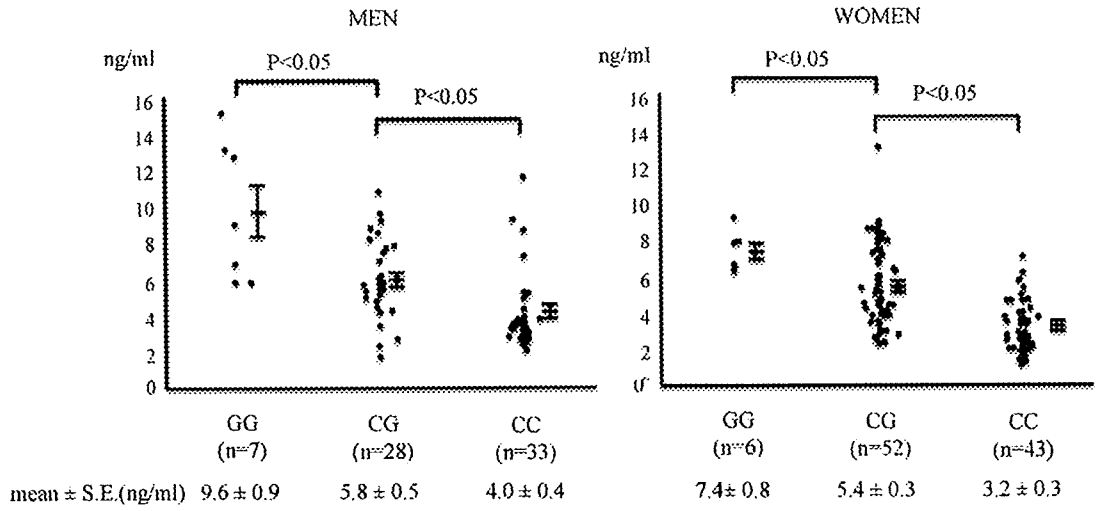

Figure 2 Plasma resistin concentration and resistin −420C > G genotype in Japanese men and women.

Association of the −420C > G SNP with plasma resistin concentration in Japanese men and women

We examined the relation between the plasma resistin concentration and the −420C > G SNP using the samples of healthy volunteers in Iwate prefecture. Fig. 2 shows plasma concentration of resistin in men and women according to the −420GG, CG and CC genotype, respectively. Plasma resistin concentration was tended to be higher in men than in women (5.3 ± 0.3 vs. 4.6 ± 0.3; $P = 0.055$). In both men and women, plasma resistin concentration was significantly high in order of those with the −420GG, CG and CC genotype.

Discussion

This study used the 2005 Japanese definition of metabolic syndrome to diagnose and study 2968 individuals from the general population. We defined subjects having none of the components of this syndrome as controls, and thus obtained 424 metabolic syndrome subjects and 765 control subjects for the case–control study. After a thorough analysis of SNPs in the full-length resistin gene, we selected five tagging SNPs to predict haplotypes and identified one linkage disequilibrium block.

We then demonstrated that the GAC and CGC haplotypes had opposite effects on metabolic syndrome susceptibility, the former being associated with an increased risk of metabolic syndrome and the latter with a decreased risk. However, this was only true for men and there was no such association for women. We also showed that the −358A and −420G alleles, which were in linkage disequilibrium, and the +299A allele, which was not linked with the other alleles, were all associated with an increased risk of metabolic syndrome. Furthermore, the −420G allele was significantly correlated with high diastolic blood pressure, high serum triglyceride, low HDL-cholesterol and high HOMA-IR levels. Interestingly, the serum level of adiponectin (a hormone involved in insulin resistance and atherosclerosis) was also correlated with the allele, implying that these genetic variations might promote metabolic syndrome.

Previous studies on the association of resistin gene variants with obesity and type 2 diabetes have yielded conflicting results. Sentinelli et al. found no significant association of resistin gene variants in European subjects with type 2 diabetes or obesity compared to controls [15]. Osawa et al. also failed to detect an association between the −167C > T, +157C > T, and +299G > A SNPs of this gene and type 2 diabetes [16]. However, Engert et al. found an association between SNPs in the resistin gene promoter region and obesity in Canadian and Scandinavian populations [13]. Subsequently, Osawa et al. demonstrated that the CG + GG genotype of the −420C > G SNP of resistin gene is significantly associated with type 2 diabetes in Japanese subjects [21]. Additionally, they showed that this variation enhanced resistin gene promoter activity through specific binding of Sp1/3, implying that the −420C > G SNP is a causative variant [21]. We found significant associations between the prevalence of metabolic syndrome in Japanese men and resistin SNPs and haplotypes, with markedly lower *P*-values than those reported to date. Such strong associations might be due to the large sample size and the

selection of a cohort that is representative of urban Japanese populations.

Gender differences in the prevalence of metabolic syndrome have been reported [22]. Previous studies have shown that visceral fat is highly responsive to androgens, suggesting that a gender difference in the etiology of this syndrome may exist. There is also a gender difference in plasma adiponectin levels [23], and the results from this genetic study are compatible with these observations.

In mice, previous observations have suggested that resistin plays a role in insulin resistance and glucose metabolism. Banerjee et al. showed that mice with the null allele of this gene have improved glucose tolerance compared with control littermates when fed a high-fat diet [9]. This change was paralleled by decreased hepatic glucose production due to decreased gluconeogenesis. Enzymes involved in gluconeogenesis, such as glucose-6-phosphate (G6P) and phosphoenolpyruvate carboxykinase (PEPCK), had decreased activity. This reduction in activity was partly due to AMPK activation as resistin deficiency led to AMPK phosphorylation. These results suggest that resistin may enhance hepatic gluconeogenesis, presumably by antagonizing adiponectin, which inhibits enzymes involved in gluconeogenesis through AMPK activation. However, in humans, the role of resistin in insulin resistance is unclear. Fehmann and Heyn reported that plasma resistin levels are not different in type 1 and type 2 diabetes [12] and Menzaghi et al. showed the no relation to insulin resistance [24]. However, a small observational human study has indicated that serum resistin levels negatively correlate with HDL-cholesterol level, which is a component of metabolic syndrome [25].

We found that certain resistin gene variants correlated with metabolic syndrome in Japanese men. However, two limitations of this study should be noted. (1) The Japanese criteria for metabolic syndrome differ from those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP-ATP III). Because of the cut-off value for waist circumference, a relatively small number of women were included in this study. However, the odds ratio for metabolic syndrome susceptibility in women was almost equal to the value obtained with the NCEP-ATP III criteria (data not shown). (2) The study cohort consisted predominantly of elderly Japanese men and women living in urban areas with a temperate climate. Therefore, these results need to be confirmed in other cohorts.

In summary, we found that the G allele of the −420C > G SNP of the resistin gene increased susceptibility to metabolic syndrome and correlated with the clinical traits of this syndrome. This SNP was also associated with lower serum adiponectin levels, suggesting a possible functional relevance of the *resisitin gene* in metabolic syndrome. Taken together with previous results, resistin may increase the susceptibility of metabolic syndrome by modulating lipid metabolism and adiponectin secretion from adipocytes. Further investigations are needed to confirm this hypothesis.

Conflict of interest

Authors have no competing interest in this article.

Acknowledgments

We are grateful to the following people for their support of our population survey: Dr. Yasushi Kotani, President; Dr. Katsuyuki Kawanishu, Head of the Committee for the city health check service; and other members of the Suita City Medical Association. We also thank the members of Satsuki-Junyu-kai for their cooperation and support of our survey of risk factors and prevention of cardiovascular disease. We also thank Mrs. Yae Takahashi and the members of Yahaba town.

Sources of funding: This study was supported by the Program for the Promotion of Fundamental Studies in Health Science of the Organization for Pharmaceutical Safety and Research of Japan, the National Institute of Biomedical Innovation (NIBIO) of Japan and the Research Grant for Cardiovascular Diseases from the Ministry of Health, Labour and Welfare and the Research Grant of Cardiovascular Diseases (18C-7) from the Ministry of Health, Labour and Welfare.

References

[1] Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on detection, evaluation, and treatment of high blood cholesterol in adults (Adult Treatment Panel III). *JAMA* 2001;285:2486–97.

[2] Grundy SM, Brewer Jr HB, Cleeman JI, Smith Jr SC, Lenfant C. Definition of metabolic syndrome: report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. Circulation 2004;109:433–8.

[3] Grundy SM. Metabolic syndrome: connecting and reconciling cardiovascular and diabetes worlds. J Am Coll Cardiol 2006;47:1093–100.

[4] Orchard TJ, Temprosa M, Goldberg R, Haffner S, Ratner R, Marcovina S, et al. The effect of metformin and intensive

lifestyle intervention on the metabolic syndrome: the Diabetes Prevention Program randomized trial. Ann Intern Med 2005;142:611–9.
[5] Ouchi N, Kihara S, Arita Y, Maeda K, Kuriyama H, Okamoto Y, et al. Novel modulator for endothelial adhesion molecules: adipocyte-derived plasma protein adiponectin. Circulation 1999;100:2473–6.
[6] Haugen F, Jorgensen A, Drevon CA, Trayhurn P. Inhibition by insulin of resistin gene expression in 3T3-L1 adipocytes. FEBS Lett 2001;507:105–8.
[7] Day C. Thiazolidinediones: a new class of antidiabetic drugs. Diabet Med 1999;16:179–92.
[8] Steppan CM, Bailey ST, Bhat S, Brown EJ, Banerjee RR, Wright CM, et al. The hormone resistin links obesity to diabetes. Nature 2001;409:307–12.
[9] Banerjee RR, Rangwala SM, Shapiro JS, Rich AS, Rhoades B, Qi Y, et al. Regulation of fasted blood glucose by resistin. Science 2004;303:1195–8.
[10] Savage DB, Sewter CP, Klenk ES, Segal DG, Vidal-Puig A, Considine RV, et al. Resistin/Fizz3 expression in relation to obesity and peroxisome proliferator-activated receptor-gamma action in humans. Diabetes 2001;50: 2199–202.
[11] Nagaev I, Smith U. Insulin resistance and type 2 diabetes are not related to resistin expression in human fat cells or skeletal muscle. Biochem Biophys Res Commun 2001;285: 561–4.
[12] Fehmann HC, Heyn J. Plasma resistin levels in patients with type 1 and type 2 diabetes mellitus and in healthy controls. Horm Metab Res 2002;34:671–3.
[13] Engert JC, Vohl MC, Williams SM, Lepage P, Loredo-Osti JC, Faith J, et al. 5′ flanking variants of resistin are associated with obesity. Diabetes 2002;51:1629–34.
[14] Conneely KN, Silander K, Scott LJ, Mohlke KL, Lazaridis KN, Valle TT, et al. Variation in the resistin gene is associated with obesity and insulin-related phenotypes in Finnish subjects. Diabetologia 2004;47:1782–8.
[15] Sentinelli F, Romeo S, Arca M, Filippi E, Leonetti F, Banchieri M, et al. Human resistin gene, obesity, and type 2 diabetes: mutation analysis and population study. Diabetes 2002;51:860–2.
[16] Osawa H, Onuma H, Murakami A, Ochi M, Nishimiya T, Kato K, et al. Systematic search for single nucleotide polymorphisms in the resistin gene: the absence of evidence for the association of three identified single nucleotide polymorphisms with Japanese type 2 diabetes. Diabetes 2002;51:863–6.
[17] Wang H, Chu WS, Hemphill C, Elbein SC. Human resistin gene: molecular scanning and evaluation of association with insulin sensitivity and type 2 diabetes in Caucasians. J Clin Endocrinol Metab 2002;87:2520–4.
[18] Ma X, Warram JH, Trischitta V, Doria A. Genetic variants at the resistin locus and risk of type 2 diabetes in Caucasians. J Clin Endocrinol Metab 2002;87:4407–10.
[19] Ochi M, Osawa H, Onuma H, Murakami A, Nishimiya T, Shimada F, et al. The absence of evidence for major effects of the frequent SNP +299G > A in the resistin gene on susceptibility to insulin resistance syndrome associated with Japanese type 2 diabetes. Diabetes Res Clin Pract 2003;61:191–8.
[20] Matsuzawa Y. Metabolic syndrome—definition and diagnostic criteria in Japan. J Atheroscler Thromb 2005;12:301.
[21] Osawa H, Yamada K, Onuma H, Murakami A, Ochi M, Kawata H, et al. The G/G genotype of a resistin single-nucleotide polymorphism at −420 increases type 2 diabetes mellitus susceptibility by inducing promoter activity through specific binding of Sp1/3. Am J Hum Genet 2004;75:678–86.
[22] Reynolds K, He J. Epidemiology of the metabolic syndrome. Am J Med Sci 2005;330:273–9.
[23] Bottner A, Kratzsch J, Muller G, Kapellen TM, Bluher S, Keller E, et al. Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. J Clin Endocrinol Metab 2004;89:4053–61.
[24] Menzaghi C, Coco A, Salvemini L, Thompson R, De Cosmo S, Doria A, et al. Heritability of serum resistin and its genetic correlation with insulin resistance-related features in nondiabetic Caucasians. J Clin Endocrinol Metab 2006;91:2792–5.
[25] Chen CC, Li TC, Li CI, Liu CS, Wang HJ, Lin CC. Serum resistin level among healthy subjects: relationship to anthropometric and metabolic parameters. Metabolism 2005;54:471–5.

Contents lists available at ScienceDirect

Diabetes Research and Clinical Practice

journal homepage: www.elsevier.com/locate/diabres

Association study of 11β-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort

Yoshihiro Miyamoto [a,*], Hiroko Morisaki [b], Itaru Yamanaka [b], Yoshihiro Kokubo [c], Hiroaki Masuzaki [d], Akira Okayama [c], Hitonobu Tomoike [e], Kazuwa Nakao [d], Tomonori Okamura [c], Yasunao Yoshimasa [a], Takayuki Morisaki [b]

[a] Department of Atherosclerosis and Diabetes, National Cardiovascular Center, Osaka, Japan
[b] Department of Bioscience, National Cardiovascular Center Research Institute, Osaka, Japan
[c] Department of Preventive Cardiology, National Cardiovascular Center, Osaka, Japan
[d] Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Kyoto, Japan
[e] National Cardiovascular Center Hospital, Osaka, Japan

ARTICLE INFO

Article history:
Received 13 September 2008
Received in revised form
11 May 2009
Accepted 20 May 2009
Published on line 16 June 2009

Keywords:
11β-Hydroxysteroid dehydrogenase type 1
Metabolic syndrome
Single nucleotide polymorphism
Japanese
Haplotype

ABSTRACT

11β-Hydroxysteroid dehydrogenase type 1 (11β-HSD1), one of the isoforms of the 11β-hydroxysteroid dehydrogenase enzymes, acts as an oxo-reductase to reactivate cortisone to cortisol, plays a critical role in tissue-specific corticosteroid reactions, and is therefore a key molecule associated with the development of metabolic syndrome. We investigated whether variations in the 11β-HSD1 gene correlated with metabolic syndrome. We performed case–control study using a population-based urban Japanese cohort. Among 3005 urban residents, we examined 431 subjects diagnosed with metabolic syndrome according to the Japanese definition and 777 subjects with none of metabolic syndrome criteria as control. We genotyped three single nucleotide polymorphisms (SNPs) (+9410T>A, +17925C>T, +27447G>C) across the 11β-HSD1 gene in them and analyzed the associations of SNPs and haplotypes with metabolic syndrome. The +9410A allele showed a tendency to metabolic syndrome (OR = 1.5, 95%C.I., 1.0–2.2; P = 0.041 and Bonferroni corrected P = 0.123) without statistical significance. However, we could not find any significant association between metabolic syndrome and SNPs in the 11β-HSD1 gene. Our findings indicate that polymorphisms and haplotypes in the 11β-HSD1 gene are not significantly associated with metabolic syndrome in the Japanese population.

1. Introduction

Two isoforms of the 11β-hydroxysteroid dehydrogenase enzyme (11β-HSD), 11β-HSD type 1 (11β-HSD1) and 11β-HSD type 2 (11β-HSD2), catalyze the conversion between hormonally active cortisol and inactive cortisone [1]. 11β-HSD1 acts as an oxo-reductase that reactivates cortisone to cortisol [1] and is an abundant intracellular component in adipose tissue, liver and central nervous system [1–3]. In contrast, 11β-HSD2 is a dehydrogenase that inactivates cortisol to cortisone and is exclusively expressed in organs involved in water and electrolyte metabolism, such as the colon, kidney, sweat

* Corresponding author. Tel.: +81 6 6833 5012; fax: +81 6 6872 7486.
E-mail address: miyamoty@hsp.ncvc.go.jp (Y. Miyamoto).
0168-8227/$ – see front matter © 2009 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.diabres.2009.05.017

gland, and placenta [1,4]. This differential expression provides a mechanism for tissue-specific corticosteroid receptor activation that is independent of circulating cortisol concentrations [1,5]. Moreover, studies using animal models have shown that 11β-HSD1 increases intracellular glucocorticoid levels by converting circulating 11-dehydrocorticosterone (cortisone in humans) into active corticosterone (cortisol) through 11β-reductase in adipocytes decrease intracellular glucocorticoid levels [6–9]. In human, 11β-HSD activity in adipose tissue was positively correlated with BMI [10] and 11β-HSD1 inhibition enhances insulin sensitivity and provides a new approach to control metabolic diseases, including type 2 diabetes [11–13].

Epidemiologic studies have indicated that metabolic syndrome has become more prevalent in Western and Asian countries due to both environmental factors and lifestyle changes, such as a high-calorie diet and sedentary behavior. However, there is also evidence that certain individuals are genetically predisposed to metabolic syndrome and its related traits. Polymorphisms in the HSD11B1 gene which encodes 11β-HSD1 have been reported to be associated with type 2 diabetes [14] and hypertension [15]. In particular, Gelernter-Yaniv et al. reported the positive association of the ins4436A SNP in the HSD11B1 gene with BMI and insulin resistance in obese children [16]. However, this association has been inconsistent, probably because of differences in sample size and ethnicity [17].

In light of the possible involvement of 11β-HSD1 in metabolic syndrome, we investigated whether genetic variants of the HSD11B1 gene are associated with metabolic syndrome.

2. Methods

2.1. *Subjects and definition of metabolic syndrome*

We recruited 3655 residents on population-based cohort (Suita, Osaka Prefecture, Japan) from April 2002 to February 2004 and obtained written informed consent to study SNPs. The study design was approved by the Committee on Genetic Analysis and Gene Therapy and the ethics committee of the National Cardiovascular Center. We certify that all applicable institutional and governmental regulations concerning the ethical use of human volunteers were followed during this research. Of the 3655 participants, 3005 were included in the study because blood could be collected from them after a 12-h fast and because all three single nucleotide polymorphisms (SNPs) of the HSD11B1 gene in these subjects were successfully genotyped. According to the Japanese consensus determined by eight scientific societies including the Japanese Society of Internal Medicine, metabolic syndrome is defined as central obesity (waist circumference ≥85 cm for men and ≥90 cm for women) plus any two of the following three factors: dyslipidemia (triglycerides >1.69 mmol/l (150 mg/dl) and/or high-density lipoprotein (HDL) cholesterol <1.03 mmol/l (40 mg/dl), or lipid-lowering therapy), hypertension (systolic blood pressure (SBP) ≥130 and/or diastolic blood pressure (DBP) ≥85 mmHg, or antihypertensive therapy), and fasting plasma glucose ≥6.11 mmol/l (110 mg/dl) or previously diagnosed type 2 diabetes [18]. Subjects with none of these metabolic syndrome criteria were defined as controls. Among 3005 persons, 431 persons met the metabolic syndrome criteria, 777 persons did not meet any one of the metabolic syndrome criteria, and 1797 persons who belonged neither to metabolic syndrome nor to controls were indicated as intermediate in Table 1. The Japanese criteria for metabolic syndrome differ from those of the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III), which is considered present when at least three of the five traits including an increased waist circumference, blood pressure elevation, low HDL cholesterol, high triglycerides, and hyperglycemia. As we thought whether a 11βHSD gene was involved in the crises of the metabolic syndrome with the pathology which made visceral fat accumulation a base, we used the Japanese criertia for metabolic syndrome.

2.2. *Clinical parameters*

Blood pressure was measured after at least 10 min of rest in the sitting position. The mean values of two SBP or DBP measurements obtained by a physician using a mercury sphygmomanometer (recorded >3 min apart) were used for analysis. After 12 h of fasting, blood samples were collected, and total cholesterol, HDL-cholesterol, and triglyceride levels were measured with an autoanalyzer (Toshiba TBA-80) 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.

2.3. *Anthropometric estimates*

The participants, wearing no shoes and only underwear, were weighed on an electronic scale, and results were recorded to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using height meter with the subject standing. Waist diameters were measured to the nearest 1.0 cm at the height of the navel upon breath intake using a non-extendable linen tape measure.

2.4. *Screening and identification of SNPs in the human HSD11B1 gene*

Genomic DNA samples were isolated from peripheral leukocytes of the participants. Eight primer sets were designed to amplify the promoter and intron/exon boundaries of the HSD11B1 gene, and an initial SNP screening was performed using 48 randomly chosen DNA samples. Screening for genetic variants was performed using a denaturing HPLC method, in which the PCR products were analyzed using WAVE DNA Fragment Analysis and WAVEMAKER software 4.0 (Transgenomic Inc., Omaha, NE, USA), following the manufacturer's protocol. All detected variations were confirmed by a direct sequencing using an ABI 3700 (Applied Biosystems, Foster City, CA, USA). SNPs were genotyped using TaqMan PCR (ABI PRISM 7900HT, Applied Biosystems). The validity of the detection systems was verified prior to the large-scale study, using 48 samples that were genotyped at the initial screening.

2.5. *Estimation of haplotype frequencies and evaluation of linkage disequilibrium (LD) patterns in the HSD11B1 gene*

We estimated the frequencies of the haplotypes and the coefficient for LD (D' and r^2 value) among SNPs using Haploview software version 3.32 (http://www.broad.mit.edu/mpg/haploview/).

2.6. *Statistical analysis*

Clinical parameters were compared between metabolic syndrome and control groups using the paired t-test, and a trend analysis for clinical parameters was performed using the Tukey–Kramer HSD test. Fasting blood glucose and triglyceride levels were transformed to natural logarithms before analysis. The association between the risk haplotype and metabolic syndrome was assessed by the chi-square test using Haploview software (http://www.broad.mit.edu/haploview/haploview). This software enables a haplotype population frequency estimation and tests the significant association by Z-test. The genotypic relative risk was assessed by comparing the metabolic syndrome group with the control group and calculating the odds ratio (OR) and the 95% confidence interval (C.I.), using a logistic regression analysis after adjusting for age and sex.

All P values are two-tailed, and P values below 0.05 were considered statistically significant after Bonferroni correction. Statistical analyses were performed using JMP software, version 6.0 (SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. *Clinical features of metabolic syndrome subjects*

Table 1 shows the clinical characteristics of metabolic syndrome and control subjects. Men had a higher prevalence of metabolic syndrome than women (men, 24.2%; women, 6.1%) and metabolic syndrome subjects were older than subjects without the syndrome (age, 67.6 ± 9.5; 59.5 ± 11.5 years, respectively). Metabolic syndrome had a significantly higher body mass index (BMI), waist circumference, systolic and diastolic blood pressures, fasting glucose, HbA1c, and triglyceride levels and significantly lower HDL-cholesterol, reflecting the criteria of metabolic syndrome. Total cholesterol levels were not significantly different between the groups.

3.2. *Identification of polymorphisms in the HSD11B1 gene*

Forty-eight individuals were examined for polymorphisms in the 11β-HSD1 gene using the WAVE system. A total of seven SNPs and an insertion polymorphism were found in the gene. All eight polymorphisms were in Hardy–Weinberg equilibrium, and the seven SNPs were reported in the NCBI db SNP database (http://www.ncbi.nlm.nih.gov/SNP/). Two of the eight polymorphisms were located in the promoter region (−718T>A, −658G>A), two polymorphisms in intron 3 (+1930insA, +1972T>G), three SNPs in intron 4 (+9410T>A, +17925C>T, +27447G>C) and one SNP in the 3′UTR (+29813G>A) (Table 2).

We evaluated the linkage disequilibrium pattern among these eight polymorphisms and defined haplotypes using DNA from 48 persons. As shown in Fig. 1, one LD block consisted of SNPs from SNP-3 to SNP-8. As the D' between SNP-7 and SNP-3, -4, and -8 were all 1.00, respectively, and the r^2 between SNP-7 and SNP-3, -4, and -8 were 1.00, 1.00, and 0.899, respectively, we considered that SNP-7 captured SNP-3, -4, and -8. Taking together with their low allele frequencies of SNP-1 and -2, we used three SNPs (SNP-5, -6, and -7) for determining a haplotype of the LD block and this association study.

3.3. *Association of SNPs and haplotypes of the HSD11B1 gene with metabolic syndrome*

As shown in Table 3, the +9410T>A SNP was nominally associated with metabolic syndrome after adjusting for sex

Table 1 – Comparison of clinical parameters among metabolic syndrome, intermediate and control groups in an urban Japanese population (n = 3005).

	Control (n = 777)	Intermediate (n = 1797)	MS (n = 431)	P*
Men (n, %)	198, 25.5	841, 46.8	331, 76.8	<0.001
Age (year)	59.5 ± 11.5	68.0 ± 10.0	67.6 ± 9.5	<0.001
BMI (kg/m²)†	20.8 ± 2.3	23.0 ± 2.9	25.9 ± 2.7	<0.001
Waist (cm)†	77.3 ± 6.3	85.0 ± 8.0	93.1 ± 6.0	<0.001
SBP (mmHg)†	112.1 ± 9.9	135.2 ± 18.2	140.9 ± 16.0	<0.001
DBP (mmHg)†	71.0 ± 7.5	79.3 ± 9.4	83.6 ± 9.7	<0.001
FBG (mmol/l)†	5.02 ± 0.42	5.52 ± 1.04	6.59 ± 1.76	<0.001
HbA1c (%)†	5.2 ± 0.3	5.5 ± 0.7	6.1 ± 1.1	<0.001
T-Cho (mmol/l)	5.35 ± 0.83	5.40 ± 0.82	5.35 ± 0.92	1.000
TG (mmol/l)†	0.83 ± 0.30	1.19 ± 0.68	1.89 ± 1.00	<0.001
HDLc (mmol/l)†	1.75 ± 0.39	1.55 ± 0.39	1.28 ± 0.33	<0.001

MS: metabolic syndrome, SBP: systolic blood pressure, DBP: diastolic blood pressure, FBG: fasting blood glucose, TG: triglycerides, T-Cho: total cholesterol, HDLc: HDL cholesterol. Data are shown as the mean ± SD. The laboratory data reported in milligram per deciliter can be converted to SI units as follows: total cholesterol, HDL cholesterol: mg/dl × 0.02586 = mmol/l; triglycerides: mg/dl × 0.01129 = mmol/l; fasting blood glucose: mg/dl × 0.05556 = mmol/l.

* P-values for the comparison between MS and control groups.

† P-values for the trend among the three groups were less than 0.05.

Table 2 – Characteristics of the polymorphisms in the 11β-HSD1 gene locus.

SNP	Position genome[a]	dbSNP ID	Variation	Location	Frequency of minor alleleb[b]
1	−718	rs860185	T>A	5′flanking	0.010
2	−658		G>A	5′flanking	0.010
3	+1930		insA	Intron 3	0.104
4	+1972	rs12086634	T>G	Intron 3	0.104
5	+9410	rs2236905	T>A	Intron 4	0.073
6	+17925	rs2298930	C>T	Intron 4	0.344
7	+27447	rs932335	G>C	Intron 4	0.104
8	+29813	rs6752	G>A	Exon 6	0.115

[a] Numbers indicate locations relative to A of the ATG translation initiation codon.
[b] Based on screening results of 48 pilot samples.

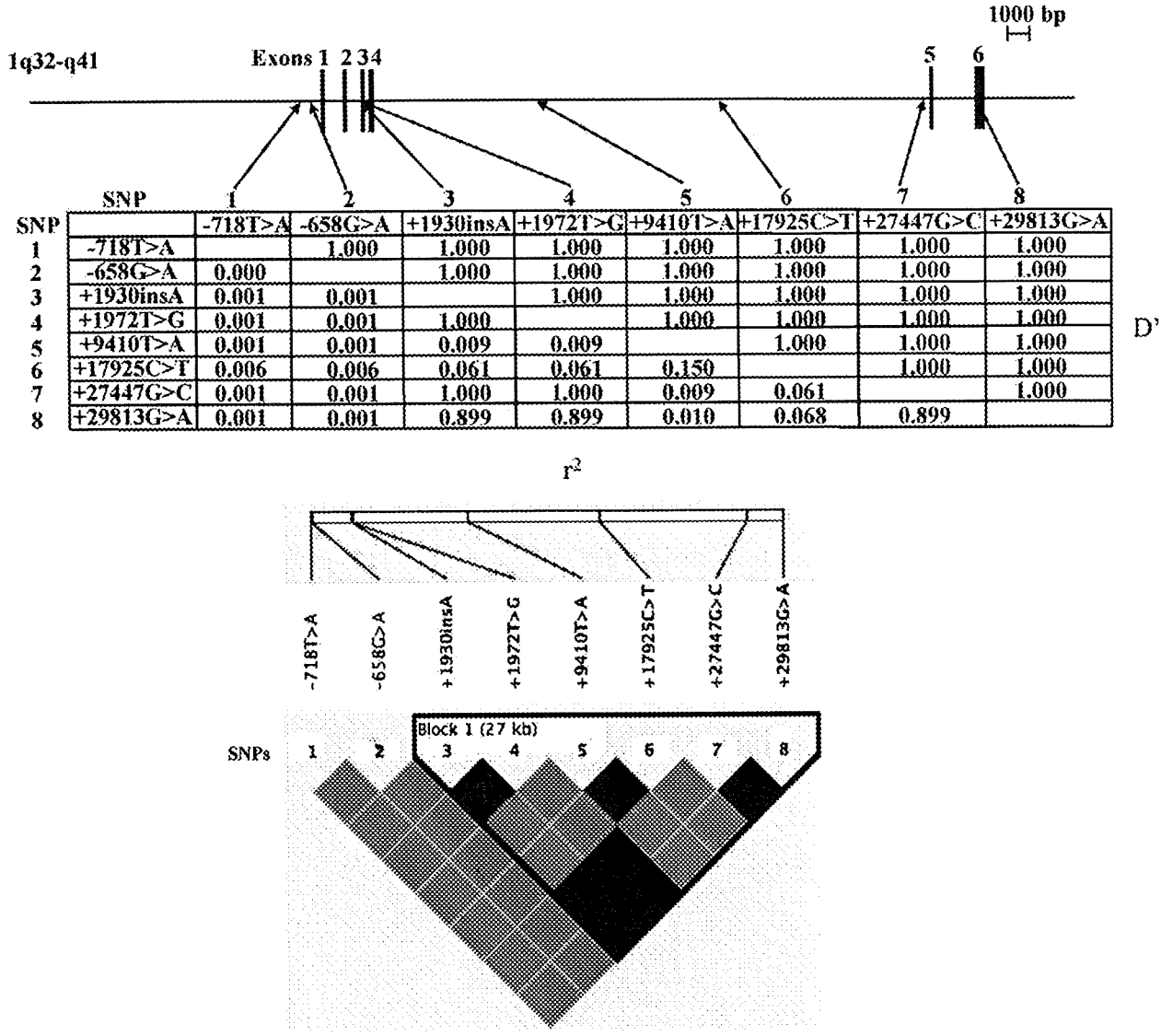

Fig. 1 – Pairwise linkage disequilibrium in 11β-HSD1 gene evaluated by D′ and r^2. Using the eight SNPs (−718T>A, −658G>A, +1930insA, +1972T>G, +9410T>A, +17925C>T, +27447G>C, +29813G>A) in the 11β-HSD1 gene, we calculated pairwise r^2 and D′ for each SNP pair and evaluated the linkage disequilibrium pattern of the 11β-HSD1 gene in 48 pilot samples. The three SNPs (+1930insA, +1972T>G and +27447G>C) are completely linked each other as both r^2 and D′ among these SNPs equal to 1.0. A bold line surrounds a haplotype block.