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Low-density lipoprotein cholesterol and non-high-density lipoprotein cholesterol and the incidence of cardiovascular disease in an urban Japanese cohort study: The Suita study

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ABSTRACT

Objective: Only a small number of population-based cohort studies have directly compared the predictive value of low-density lipoprotein cholesterol (LDL-C) and non-high-density lipoprotein cholesterol (non-HDLC) for coronary artery disease in Asian populations, such as Japan.
Methods: We performed an 11.9-year cohort study of 4694 men and women, aged 30–74 years, selected randomly from an urban general population in Japan. Baseline LDL-C levels were estimated using the Friedewald formula. The predictive values of LDL-C and non-HDLC for myocardial infarction (MI) and stroke were compared.
Results and conclusion: During the follow-up period, there were 80 incident cases of MI and 139 of stoke, comprised of 23 intracerebral hemorrhages, 85 cerebral infarctions and 31 other types of stroke. The Hazard ratio (HR) for MI was highest in the top quintile of LDL-C (HR: 3.03, 95% CI, 1.32–6.96) when male and female data were combined. The HR for MI was also highest in the top quintile of non-HDLC (HR: 2.97, 95% CI, 1.26–6.97). Analysis of trends showed a significant positive relationship between MI incidence and serum LDL-C and non-HDLC levels (both $P=0.02$). However, there was no relationship between the incidence of any subtype of stroke and either LDL-C or non-HDLC. The predictive value of LDL-C and non-HDLC for MI, assessed by calculating the differences in the −2 logarithm likelihood (−2 ln [L]) and area under the curve (AUC), were almost similar.

1. Introduction

The causal relationship between high levels of serum low-density lipoprotein cholesterol (LDL-C) and coronary artery disease (CAD) is well established [1–5]. Blood LDL-C levels are therefore the main target for lipid management in the majority of guidelines of developed countries for preventing atherosclerotic disease [3–5]. Some US cohort studies have also suggested that non-high-density lipoprotein (non-HDLC) may be a better predictor of CAD [6,7]. However, to our knowledge, only one population-based cohort study has directly compared the predictive value of these lipid markers for CAD in an Asian population [8], which have a lower incidence of coronary artery disease, but a higher risk of stroke than Western populations [9–12]. Furthermore, although it has not been shown that there is a positive relationship between the risk of any type of stroke and high serum levels of total cholesterol (TC) in the Japanese population [9,10], the effects on stroke incidence of the closely related lipid fractions, LDL-C and non-HDLC, have not been evaluated.

The purpose of this study was therefore to investigate the predictive value of LDL-C and non-HDLC for the incidence of CAD and stroke in a Japanese urban population over an 11.9-year period. Our *a priori* hypothesis was that both LDL-C and non-HDLC may be useful predictors of CAD risk, but not of stroke risk.

2. Methods

2.1. Populations

The Suita study [13,14], a cohort study of cardiovascular disease, was established in 1989 and included 12,200 Japanese urban residents of Suita City, Osaka. The participants, aged 30–79 years,

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were selected randomly from the municipality population registry. Of these, 6485 men and women had a baseline medical examination at the National Cardiovascular Center between September 1989 and March 1994 (participation rate: 53.2%). Of the 6485 participants, a total of 1791 were excluded for the following reasons: past history of coronary heart disease or stroke ($n=208$), nonperiodical participation in baseline survey ($n=79$), aged 75 or older ($n=343$), non-fasting visit ($n=153$), use of lipid-lowering agents such as statins ($n=106$), serum triglyceride $\geq$4.5 mmol/l (400 mg/dl) ($n=98$) and missing information at the baseline survey or lost to follow-up ($n=804$). The data of the remaining 4694 participants (2169 men and 2525 women) were then analyzed. Informed consent was obtained from all participants. This cohort study was approved by the Institutional Review Board of the National Cardiovascular Center.

2.2. Baseline examination

Blood samples were collected at the National Cardiovascular Center (NCVC) after the participants had fasted for at least 12 h. The samples were centrifuged immediately and a routine blood examination that included serum total cholesterol (TC), HDL cholesterol, triglyceride and glucose levels then carried out. LDL-C was estimated using the Friedewald formula [15]. Non-HDLC was calculated by subtracting HDL-C from TC.

Blood pressures were measured in triplicate on the right arm in the seated position after 5 min rest by well-trained physicians using a standard mercury sphygmomanometer. The average of the second and third measurements was used in the analyses. Hypertension was defined as either a systolic blood pressure $\geq$140 mmHg, a diastolic blood pressure $\geq$90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose $\geq$7.0 mmol/l (126 mg/dl), the use of anti-diabetic agents, or both. Height in stockings and weight in light clothing were measured. Public health nurses obtained information on the smoking, drinking and medical histories of the participants.

2.3. Endpoint determination

The participants were followed until December 31, 2005. The first step in the survey involved checking the health status of all participants by repeated clinical visits every 2 years and yearly questionnaires sent by mail or conducted by telephone. Informed consent for review of in-hospital medical records was obtained from 86.2% participants who were suspected of having had a myocardial infarction (MI) or stroke. The medical records were reviewed by registered hospital physicians or research physicians who were blinded to the baseline information.

The criteria for definite and probable MI were defined according to the criteria of the MONICA (Monitoring Trends and Determinants of Cardiovascular Disease) project [16], which requires evidence from an electrocardiogram (ECG), cardiac enzymes and/or autopsy. Stroke was defined according to the National Survey of Stroke criteria [17], which requires the rapid onset of a constellation of neurological deficits lasting at least 24 h or until death. The strokes were classified as either ischemic stroke (thrombotic or embolic), intracerebral hemorrhage, subarachnoid hemorrhage or undetermined type. A definite stroke was defined by autopsy or on the basis of diagnostic imaging, such as computed tomography or magnetic resonance imaging.

Cases with typical clinical symptoms, detected in the clinical visit during follow-up surveillance, but without informed consent for an in-hospital medical records survey, were defined as possible MI or stroke. Furthermore, to complete the surveillance for fatal MI and stroke, we conducted a systematic search for death certificates. All death certificates in Japan are forwarded to the Ministry of Health, Welfare, and Labor and coded for National Vital Statistics. We classified fatal MI and stroke listed on the death certificate, but not registered on our surveillance system, as possible MI and stroke.

2.4. Statistical analysis

Sex-specific analysis was performed. We set the cut-off points for serum LDL-C and non-HDLC according to the quintile ranges. For baseline characteristics, analysis of variance for means or Chi-square tests for proportions were used. The multivariable-adjusted hazard ratio (HR) of LDL-C and non-HDLC for MI or stroke was calculated using proportional hazards model adjusted for age, hypertension, diabetes, HDL-C, body mass index (BMI), smoking (never-smoked; ex-smoker; current smoker) and drinking (never-drank; ex-drinker; regular drinker). Sex-combined analysis with further adjustment for sex was also carried out.

Separate models with LDL-C or non-HDLC levels as ordinal variables (median of LDL-C or non-HDLC quintile) were fitted to the other risk factor adjusted models (test for trend). The differences between the -2 logarithm likelihood (-2 ln [L]) in each lipid added model and the -2 ln [L] in other risk factor adjusted models were calculated. These differences had an approximate χ^2 distribution with 1 d.f. These χ^2 values assess which lipid had the greatest predictive value in other risk factor adjusted models. The ability to predict which people developed cardiovascular disease was also assessed by calculating the area under the receiver-operating characteristic (ROC) curve (AUC). This curve showed the predictive probability of the variables using logistic regression analysis and the same covariates used in the multivariable model of test for trend. Furthermore, the predictive values of the ratio of LDL-C to HDL-C (LDL-C/HDL-C) and the ratio of non-HDLC to HDL-C (non-HDLC/HDL-C) for myocardial infarction (MI) and stroke were also compared.

All confidence intervals were estimated at the 95% level and significance was set at a P value of <0.05. The Statistical Package for the Social Sciences (SPSS Japan Inc. version 15.0J, Tokyo, Japan) was used for all the analyses.

3. Results

The mean and standard deviation of serum LDL-C in the baseline survey was 3.23 $\pm$ 0.82 mmol/l (124.9 $\pm$ 31.7 mg/dl) in men and 3.49 $\pm$ 0.90 mmol/l (134.8 $\pm$ 34.9 mg/dl) in women. The mean baseline serum non-HDLC was 3.90 $\pm$ 0.89 mmol/l (151.1 $\pm$ 34.5 mg/dl) in men and 4.01 $\pm$ 1.01 mmol/l (155.2 $\pm$ 39.1 mg/dl) in women.

Table 1 shows the baseline characteristics of the participants in each LDL-C quintile. In both sexes, there were significant differences in the mean values for age, non-HDLC, HDL-C and BMI. These variables, with the exception of HDL-C, tended to be higher in the higher LDL-C groups. Serum HDL-C levels were lower in the higher LDL-C groups. There was no significant difference in the prevalence of hypertension and diabetes in the quintiles for men, whereas the prevalence of these conditions in women was higher in the higher LDL-C groups. In both sexes, the proportion of current drinkers was lower in the higher LDL-C groups, whereas the proportion of current smokers was highest in the lowest LDL-C group. The relationships between non-HDLC quintiles and the above-mentioned baseline characteristics were almost similar (data not shown in the table).

The total person-years studied was 56,196 (25,420 for men and 30,776 for women), with a mean follow-up period of 11.9 years. During the follow-up period, there were 80 incident cases of MI (41 definite and 39 probable MIs) and 139 of stoke (102 definite and 37

Table 1
Sex-specific mean and prevalence of risk characteristics at baseline in an 11.9-year prospective study of 4694 Japanese men and women

LDL cholesterol quintiles	Q1	Q2	Q3	Q4	Q5	*P*-values
Men						
Numbers	447	435	427	438	422	
LDL cholesterol (Stratum Mean), mmol/l	2.13	2.80	3.22	3.66	4.40	
Age, year	54.0 (12.7)	53.8 (12.6)	52.5 (12.4)	54.7 (12.1)	55.6 (11.0)	0.005
Non-HDL cholesterol, mmol/l	2.84 (0.52)	3.44 (0.39)	3.87 (0.34)	4.31 (0.32)	5.13 (0.56)	<0.001
HDL cholesterol, mmol/l	1.33 (0.39)	1.29 (0.36)	1.29 (0.32)	1.26 (0.30)	1.21 (0.28)	<0.001
BMI, kg/m^2	22.1 (2.9)	22.6 (2.8)	22.9 (2.8)	23.2 (2.6)	23.4 (2.7)	<0.001
Hypertension, %	29.5	27.4	30.4	31.3	33.6	0.364
Diabetes, %	8.1	4.6	4.4	4.6	5.9	0.091
Drinking						
Usual/ex-/never-, %	81.9/2.7/15.4	78.2/2.8/19.1	79.6/1.6/18.7	71.7/5.3/23.1	70.4/4.7/24.9	<0.001
Smoking						
Current/ex-/never-, %	59.3/25.5/15.2	55.4/26.9/17.7	46.6/31.1/22.2	46.6/31.1/22.4	48.1/31.8/20.1	0.002
Women						
Numbers	524	498	513	498	492	
LDL cholesterol (Stratum Mean), mmol/l	2.33	2.98	3.44	3.92	4.82	
Age, year	45.5 (11.4)	49.9 (11.9)	52.7 (11.3)	56.3 (10.6)	57.8 (9.1)	<0.001
Non-HDL cholesterol, mmol/l	2.77 (0.42)	3.47 (0.32)	3.96 (0.31)	4.50 (0.32)	5.46 (0.71)	<0.001
HDL cholesterol, mmol/l	1.54 (0.36)	1.49 (0.36)	1.48 (0.35)	1.45 (0.33)	1.40 (0.31)	<0.001
BMI, kg/m^2	21.0 (2.7)	21.8 (3.2)	22.3 (3.3)	22.6 (3.2)	23.2 (3.3)	<0.001
Hypertension, %	12.8	19.3	23.4	29.9	37.8	<0.001
Diabetes, %	1.5	2.8	3.1	4.0	4.7	0.050
Drinking						
Usual/ex-/never-, %	41.8/2.3/55.9	36.5/1.0/62.4	32.7/1.4/65.9	28.3/1.8/69.9	29.1/1.6/69.3	<0.001
Smoking						
Current/ex-/never-, %	16.4/4.6/79.0	12.7/3.8/83.5	9.6/2.1/88.3	10.8/3.4/85.7	11.6/3.7/84.8	0.015

HDL means high-density lipoprotein. LDL means low-density lipoprotein. S.D. means standard deviations. Brackets indicate standard deviation. Analysis of variance was used for comparisons of multiple group means and the Chi-square test was used to compare frequencies.

probable strokes), comprised of 23 intracerebral hemorrhages, 85 cerebral infarctions and 31 other types of stroke.

Table 2 shows the number of incident cases and multivariable-adjusted HRs for MI and cerebral infarction stratified by LDL-C quintile. In women, the bottom and second quintiles and the third and fourth quintiles were combined into two categories due to the small number of cardiovascular events. In both sexes, the HR for MI was highest in the top quintile of LDL-C, although the value in women was not statistically significant (HR 3.73; 95% CI 1.25–11.1 for men: HR 1.78; 95% CI 0.66–4.77 for women). In the test for trend, serum LDL-C showed a significant positive association with MI when the data from men and women were combined

Table 2
The numbers of cases and multivariable-adjusted HRs and 95% C.I.s for myocardial infarction and cerebral infarction according to serum LDL cholesterol level in an 11.9-year prospective study of 4694 Japanese men and women

LDL cholesterol quintiles	LDL-C range (mmol/l)	No. of persons	Person-years	Myocardial infarction			Cerebral infarction		
				No. of events	HR[a]	95% C.I.	No. of events	HR[a]	95% C.I.
Men									
Q1	<2.54	447	5,129	4	1.00		14	1.00	
Q2	2.54–3.03	435	5,122	15	3.56	1.18, 10.8	9	0.61	0.26, 1.42
Q3	3.04–3.43	427	4,945	9	2.60	0.80, 8.5	15	1.31	0.63, 2.72
Q4	3.44–3.90	438	5,201	10	2.25	0.70, 7.2	13	0.90	0.42, 1.94
Q5	3.91–	422	5,023	18	3.73	1.25, 11.1	6	0.42	0.16, 1.10
					P for trend	0.08		*P* for trend	0.22
Women									
Q1 + Q2[b]	<3.21	1022	12,473	6	1.00		7	1.00	
Q3 + Q4[b]	3.22–4.22	1011	12,279	5	0.45	0.14, 1.49	11	0.82	0.31, 2.15
Q5	4.23	492	6,023	13	1.78	0.66, 4.77	10	1.13	0.42, 3.02
					P for trend	0.14		*P* for trend	0.88
Men and women combined									
Q1		971	11,548	7	1.00		19	1.00	
Q2		933	11,176	18	2.37	0.97, 5.61	11	0.53	0.25, 1.12
Q3	[c]	940	11,102	11	1.57	0.61, 4.08	18	0.95	0.49, 1.82
Q4		936	11,323	13	1.40	0.56, 3.55	21	0.84	0.44, 1.59
Q5		914	11,046	31	3.03	1.32, 6.96	16	0.63	0.32, 1.24
					P for trend	0.02		*P* for trend	0.47

LDL means low-density lipoprotein.

[a] HR means hazard ratio and 95% C.I. means 95% confidence interval. The HR was adjusted for age, body mass index, diabetes, HDL cholesterol, cigarette smoking category and alcohol intake category by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] These groups were combined due to small number of cardiovascular event. The cut-off points were 2.73 between Q1 and Q2, and 3.68 between Q3 and Q4, respectively.

[c] Sex-specific quintiles were used for analysis.

Table 3
The numbers of cases and multivariable-adjusted HRs and 95% C.I.s for myocardial infarction and cerebral infarction according to serum non-HDL cholesterol level in an 11.9-year prospective study of 4694 Japanese men and women

Non-HDL cholesterol quintiles	Non-HDLC range (mmol/l)	No. of persons	Person-years	Myocardial infarction			Cerebral infarction		
				No. of events	HR[a]	95% C.I.	No. of events	HR[a]	95% C.I.
Men									
Q1	<3.18	445	5,123	6	1.00		11	1.00	
Q2	3.18–3.68	450	5,195	14	2.34	0.89, 6.16	13	1.21	0.54, 2.73
Q3	3.69–4.12	426	5,077	7	1.21	0.40, 3.64	12	1.26	0.54, 2.91
Q4	4.13–4.63	428	5,041	10	1.49	0.53, 4.16	11	0.97	0.41, 2.31
Q5	4.64	420	4,982	19	2.61	1.00, 6.80	10	0.98	0.40, 2.40
					P for trend	0.12		*P* for trend	0.79
Women									
Q1 + Q2[b]	<3.70	1043	12,821	4	1.00		7	1.00	
Q3 + Q4[b]	3.71–4.87	1010	12,205	7	0.76	0.21, 2.72	11	0.67	
Q5	4.88	472	5,750	13	1.77	0.50, 6.25	10	0.80	
					P for trend	0.10		*P* for trend	
Men and women combined									
Q1		998	11,931	7	1.00		15	1.00	
Q2		940	11,208	17	2.35	0.97, 5.69	16	1.03	0.50, 2.10
Q3	[c]	947	11,412	11	1.38	0.53, 3.60	14	0.83	0.40, 1.76
Q4		917	10,911	13	1.40	0.55, 3.57	20	1.03	0.51, 2.06
Q5		892	10,732	32	2.97	1.26, 6.97	20	0.99	0.48, 2.03
					P for trend	0.02		*P* for trend	0.96

HDL means high-density lipoprotein.

[a] HR means hazard ratio and 95% C.I. means 95% confidence interval. The HR was adjusted for age, body mass index, hypertension, diabetes, HDL cholesterol, cigarette smoking category and alcohol intake category by a Cox proportional hazard model. Sex was also adjusted in the men and women combined model.

[b] These groups were combined due to small number of cardiovascular event. The cut-off points were 3.21 between Q1 and Q2, and 4.26 between Q3 and Q4, respectively.

[c] Sex-specific quintiles were used for analysis.

($P=0.02$). A similar trend was observed when the endpoint was limited to definite MIs by the criteria of the MONICA project ($P=0.01$, data not shown in the table). The incidence for cerebral infarction was not related to LDL-C levels in either sex. The incidences of intra-cerebral hemorrhage, other types of stroke and total stroke were also not associated with LDL-C levels (data not shown in the table).

Table 3 shows the results stratified by non-HDLC. The HR for MI was highest in the top quintile of non-HDLC in both sexes, although in women the value did not reach statistical significance (HR 2.61; 95% CI 1.00–6.8 for men: HR 1.77; 95% CI 0.50–6.25 for women). In men, the HR for MI was highest in the top quintile of non-HDLC (HR 2.61; 95% CI 1.00–6.80). In the test for trend, serum non-HDLC showed a significant positive association with MI when the data of men and women were combined ($P=0.02$). A similar trend was observed when the endpoint was limited to define MIs ($P=0.01$, data not shown in the table). The incidence of cerebral infarction was not associated with non-HDLC levels in either sex. The other types of stroke and total stroke were also not associated with non-HDLC level (data not shown in the table).

To determine the predictive values of LDL-C and non-HDLC, the difference between the −2 ln [L] of model including each lipid and the −2 ln [L] of other variable-adjusted models was calculated. The χ^2 values for LDL-C and non-HDLC were almost the same at 5.71 ($P=0.02$) for LDL-C and 5.49 ($P=0.02$) for non-HDLC. Furthermore, the AUC of the ROC curves based on predictive probability targeting for MI were also estimated. The AUC of LDL-C and non-HDLC were the same at 0.82.

We calculated the hazard ratios of LDL-C/HDL-C and non-HDLC/HDL-C, and compared the predictive values of these for the incidence of MI and stroke. Both ratios were significantly associated with the increased risk for MI but not with any types of stroke. The multivariable HRs of LDL-C/HDL-C and non-HDLC/HDL-C for MI were 1.32 [95% CI, 1.07–1.61] and 1.25 [95% CI, 1.07–1.47], respectively. Furthermore, the χ^2 values between the −2 ln (L) of each lipid added model and non-added model for LDL-C/HDL-C and non-HDLC/HDL-C were almost the same at 7.34 ($P=0.01$) for LDL-C/HDL-C and 7.06 ($P=0.01$) for non-HDLC/HDL-C. The AUC of the ROC curves based on predictive probability were also the same. Apparently, because non-HDLC/HDLC was expressed as [(TC/HDLC) − 1], the HR and predictive value for TC/HDLC were just the same as those of non-HDLC/HDLC.

When the participants were divided in two groups using the median value of serum triglycerides (1.12 mmol/l, 99 mg/dl), the results of all the analyses listed above were similar.

4. Discussion

This 11.9-year cohort study of a Japanese urban population showed a positive association between serum LDL-C or non-HDLC levels and increased risk of MI, but not with any type of stroke. Furthermore, we found there was no substantial difference in the predictive value for MI incidence between LDL-C and non-HDLC. To our knowledge, this is the first cohort study in an urban Japanese population on the relationship between serum lipids and cardiovascular events.

The role of LDL-C in the development of atherosclerosis and the beneficial effect of LDL-C lowering therapy are well established, especially in Western populations [1–4] Our study indicated there is also a positive relationship between serum LDL-C and CAD events in community-dwelling Japanese with no history of cardiovascular disease or use of lipid-lowering agents, such as statins. A recent large clinical trial in Japan [18], the Management of Elevated Cholesterol in the Primary Prevention Group of Adult Japanese (MEGA study), also have shown an 18% reduction in mean LDL-C (from 4.05 mmol/l to 3.31 mmol/l) was associated with a 33% decreased risk for CAD. These results suggested strongly that management of serum LDL-C levels is as effective for reducing CAD in Japan as it is in Western countries.

Non-HDLC levels are thought to be an alternative predictor that can substitute for LDL-C in patients with hypertriglycemia

[3]. Non-HDLC reflects the total cholesterol concentration of all atherogenic lipoproteins. Several previous studies in US communities [6,7,9,19,20] or patients with type 2 diabetes [21,22] showed that the non-HDLC level was a stronger predictor for CAD risk than LDL-C. In the Lipid Research Clinics Program Follow-up Study [6], differences of 0.78 mmol/l (30 mg/dl) in non-HDLC and LDL-C levels corresponded to increases in CVD risk of 19% and 15% in men, and 11% and 8% in women, respectively. In contrast, Chien et al. showed that the hazard ratio of the top quintile and area under the ROC curve for CAD incidence were almost similar for LDL-C and non-HDLC in ethnic Chinese living in Taiwan [8].

Our results are consistent with the Taiwan study described above [8], which to date represents the only report from a non-Western community. As we calculated serum LDL-C levels using the Friedewald formula, our results were not applicable to the population with serum triglyceride levels equal to or greater than 4.5 mmol/l (≥400 mg/dl). However, even if the predictive values of LDL-C and non-HDLC are similar in the Japanese population, non-HDLC may be the more convenient indicator to use for primary prevention in the community. Both TC and HDL-C are included in routine biochemistry measurements because of convenience and low cost, and can be measured directly even in non-fasting serum. Accordingly, non-HDLC may be a good serum marker for risk assessment of CAD in a community-based setting.

In the present study, the positive association between serum lipids levels and MI in women was less evident than that in men. We believe it was mainly due to small number of MI in women. Continued community surveillance in Japan showed that incidence of MI for women was about one third of men [23]. In the present study, incidence of MI for women was only 0.78 per 1000 person-years. Because most MI cases (22 of 24) were post-menopausal women, the low incidence of MI in pre-menopausal women was one reason for sex-difference. However, it was difficult to perform further analysis because of small sample size of MI cases.

Similar to previous studies that have explored the relationship between TC and stroke in Japan [9,24,25], we found no association between LDL-C or non-HDLC levels and stroke events. A large meta-analysis of individual data from 61 prospective studies [26], the majority of which were from the US, European and Japanese populations, showed an absence of an independent positive association between TC or non-HDLC and ischemic and total stroke mortality. Recently, the death probability over a 10-year period due to MI and stroke have been calculated and displayed as color risk score charts by combining 10-year age, systolic blood pressure, smoking, and serum total cholesterol and glucose levels by NIPPON DATA (National Integrated Project for Prospective Observation of Non-communicable Disease and Its Trends in the Aged) Research group [27]. NIPPON DATA Risk chart for MI clearly showed the positive relationship between TC and MI, however, the risk chart for stroke showed the color gradient, which was shown death probability, for stroke was not affected by TC levels.

The lack of a relationship between TC and ischemic stroke in Japanese studies may be due to a lower prevalence of thrombotic type cortical infarctions (large-artery occlusive) than in Western populations [28], a condition that is associated with atherosclerosis secondary to hypercholesterolemia. Furthermore, the Atherosclerosis Risk in Communities (ARIC) Study also indicated that TC was associated with increased risk of non-lacunar, non-embolic stroke (thrombotic type cortical infarction), but not with lacunar or embolic stroke [29]. The effect of LDL-C or non-HDLC on ischemic stroke may be weak in populations with a low prevalence of large-artery occlusive infarctions, such as in Japan. However, a meta-analysis of randomized control trials by statin therapy has indicated a reduction of stroke [30]. Even in Japanese patients with hypercholesterolemia, statin therapy showed a non-significant but inverse association with cerebral infarction [18]. Accordingly, high serum levels of LDLC or non-HDLC should be dealt with caution as a potential risk factor for ischemic stroke.

Previous studies indicated that CAD or MI morality in Japanese people was still lower than in Westerners [9–12]. However, recently, there were evidences that serum levels of TC and LDL-C in Japanese were as high as those reported in the US population [31]. However, CAD mortality has been shown to be higher in large urbanized areas in Japan such as Tokyo and Osaka compared to the rest of Japan [32]. These two cities are among the most urbanized areas in Asia. The present study therefore provides additional evidence supporting the usefulness of LDL-C and non-HDLC as predictors of future risk for MI in screening of the urbanized Japanese population. Although in Asian countries hypertension rather than LDL-C remains the most important manageable cardiovascular risk factor [33], the present study showed that, at least in urbanized areas, lowering of LDL-C levels should also be considered as an important public health issue.

The present study had some limitations. Firstly, the single LDL-C or non-HDLC measurement at the baseline survey may have underestimated the relationship between these lipids and CAD due to regression dilution bias. Secondly, we did not measure serum apolipoprotein B (apoB), which some previous studies have shown as a stronger predictor for CAD than non-HDLC [8,20]. Furthermore, measurement of apoB is not required fasting status and is estimated to be cost-efficient [34]. Further cohort studies with measurement of apoB are needed in Japanese community-dwelling populations. Thirdly, in order to accurately compare the predictive value of non-HDLC and LDL-C, serum levels of LDL-C should be measured by direct measurement of LDL-C, rather than by the Friedewald formula. Exclusion of participants with a high serum triglyceride level (≥400 mg/dl) may reduce the predictive potential of non-HDLC. Finally, the relationship between serum lipids and cerebral infarction warrants further investigation, as we did not evaluate the effect of serum LDL-C and non-HDLC on each subtype of cerebral infarction due to small sample size, especially for thrombotic type cortical infarctions.

In conclusion, higher levels of serum LDL-C and non-HDLC are both associated with an increased risk of MI, but not with cerebral infarction in a Japanese urban population. Although the predictive value of non-HDLC for MI is almost similar to that of LDL-C calculated by the Friedewald formula, non-HDLC may be recommended as an alternative screening marker for primary prevention of CAD in the community, as it is less expensive and more convenient.

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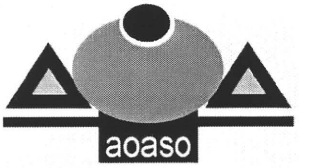

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ORIGINAL ARTICLE

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

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KEYWORDS
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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.

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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 ($n = 2968$).

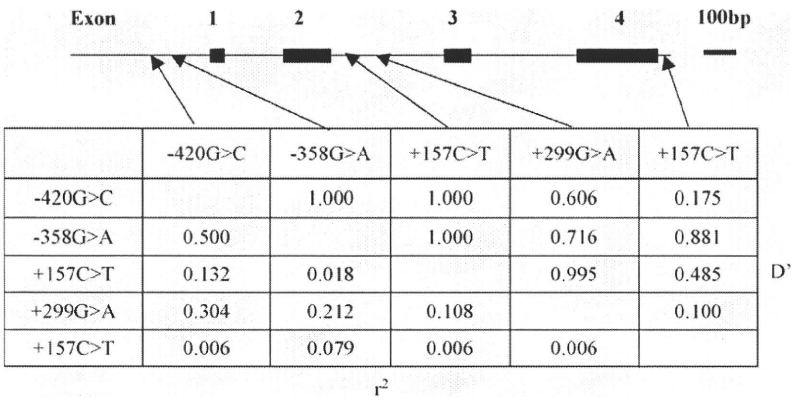

Figure 1 Using five SNPs (−420C > G, −358G > A, +157C > T, +299G > A, +1263G > C) as tags to define haplotypes, we calculated the pair-wise r^2 and D' for each SNP pair and evaluated the linkage disequilibrium pattern for the resistin gene in 2968 subjects.

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)
−420 C > 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.

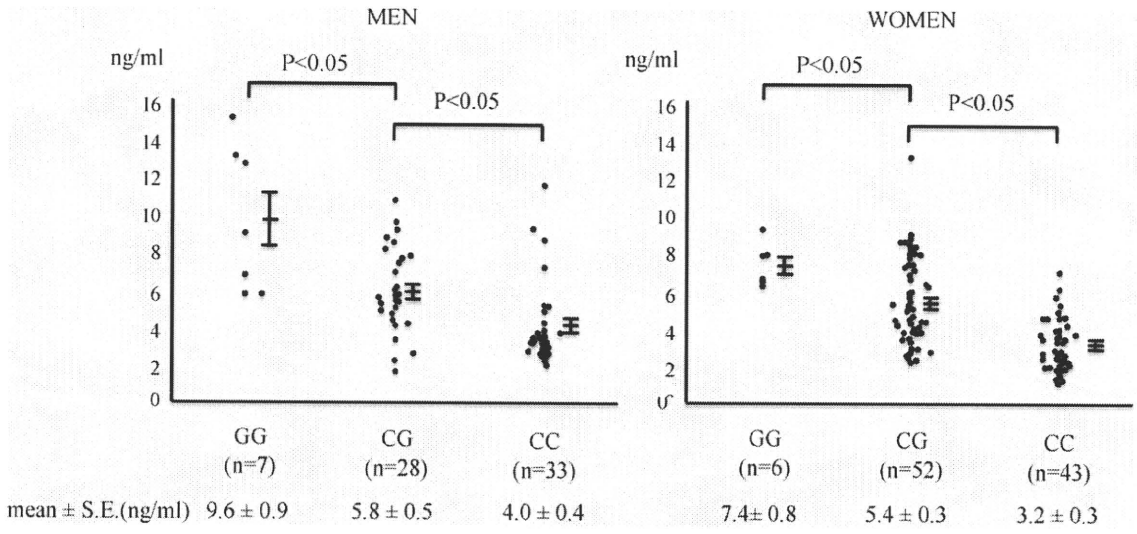

Figure 2 Plasma resistin concentration and resistin −420C > G genotype in Japanese men and women.

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.

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

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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

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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/m^2)†	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.

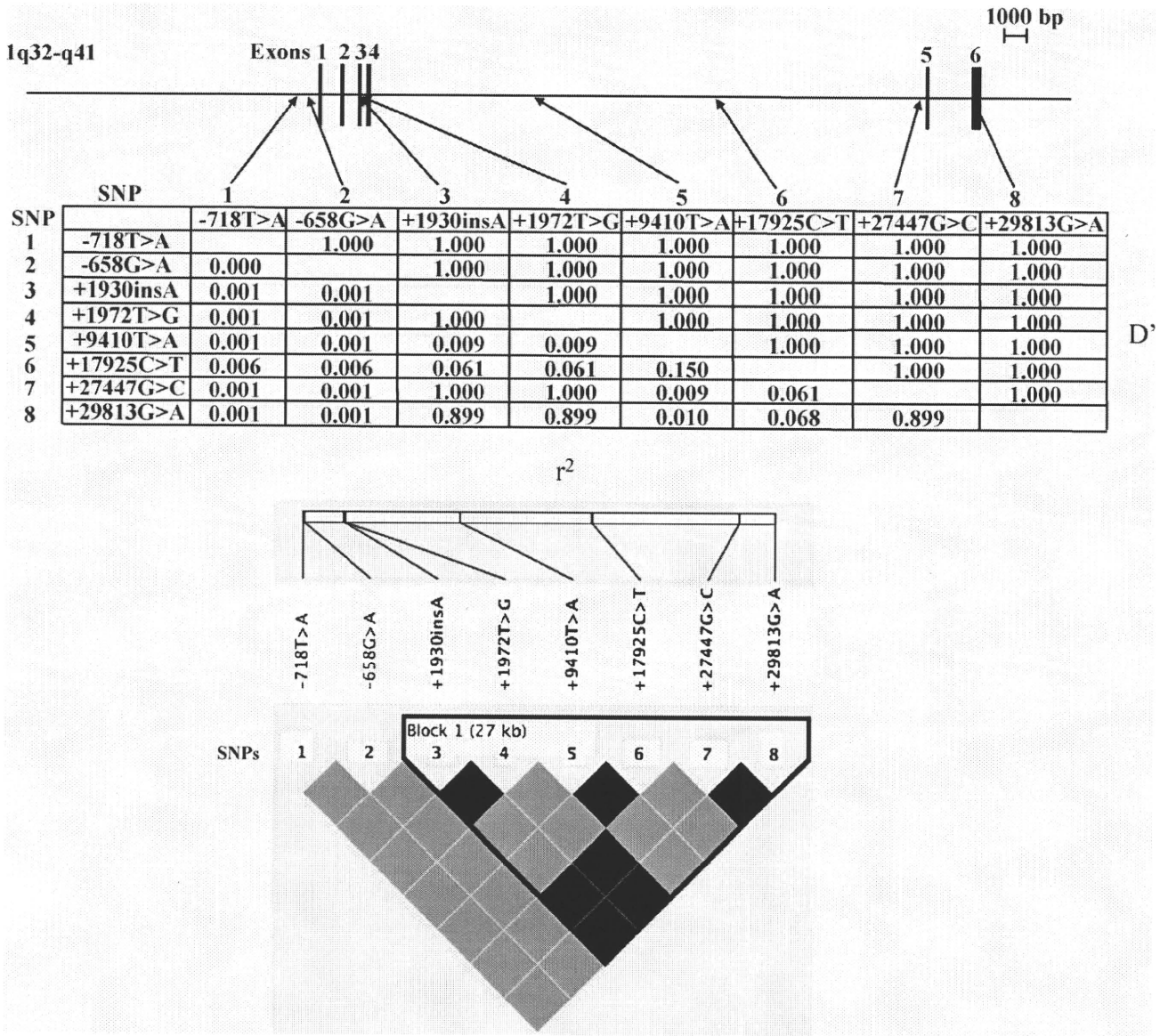
* P-values for the comparison between MS and control groups.

† 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.



SNP		-718T>A	-658G>A	+1930insA	+1972T>G	+9410T>A	+17925C>T	+27447G>C	+29813G>A
1	-718T>A		1.000	1.000	1.000	1.000	1.000	1.000	1.000
2	-658G>A	0.000		1.000	1.000	1.000	1.000	1.000	1.000
3	+1930insA	0.001	0.001		1.000	1.000	1.000	1.000	1.000
4	+1972T>G	0.001	0.001	1.000		1.000	1.000	1.000	1.000
5	+9410T>A	0.001	0.001	0.009	0.009		1.000	1.000	1.000
6	+17925C>T	0.006	0.006	0.061	0.061	0.150		1.000	1.000
7	+27447G>C	0.001	0.001	1.000	1.000	0.009	0.061		1.000
8	+29813G>A	0.001	0.001	0.899	0.899	0.010	0.068	0.899	

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.