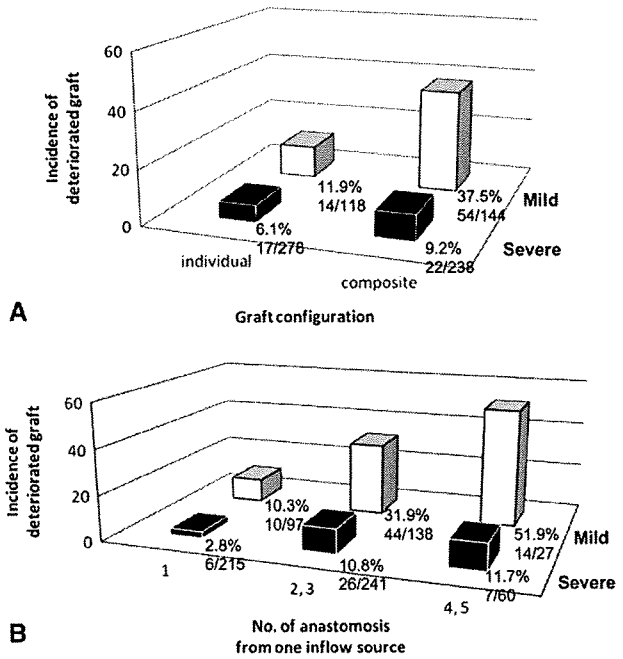

FIGURE 1. Effects of graft configuration (A) and number of distal anastomoses from single inflow source (B) on incidence of arterial graft deterioration according to severity of target coronary artery stenosis.

apparently resistant to graft deterioration. Multiple grafts originating from a single inflow source were susceptible to graft deterioration. In this study, complex design, such as composite grafting and sequential multiple grafting, appeared to have certain pitfalls, although several studies have reported excellent patency rates for such grafts.[16,17] When planning these types of grafts, selection of the target coronary artery is considered extremely important. For the target with mild stenosis, these types of grafts would result in a high failure rate, and individual grafts should be selected instead. This finding is in agreement with a previous study[18] that demonstrated reduced blood flow in a composite graft used for a less stenosed target. On the other hand, patient characteristics and postoperative medications were not related to graft deterioration. These results enhance the importance of graft design in the prevention of graft deterioration in multiple arterial revascularization procedure.

Clinical Significance of Graft Deterioration

There is no consensus regarding the clinical significance of graft deterioration, especially of a graft showing string sign. There have been several case studies[19,20] in which arterial grafts showing string sign regained patency after progression of native coronary artery stenosis. Furthermore, arterial grafts showing string sign can increase graft flow in response to a hyperemic situation, which suggests the capacity to meet the blood flow demand and to function as a bypass conduit.[21,22] Several studies, however, have suggested poor angiographic outcomes of grafts showing string sign. Kim and colleagues[12] reported that among 20 grafts initially FitzGibbon grade B, 12 grafts remained grade B and 3 grafts were occluded 3 years after surgery. Nakajima and coworkers[7] reported that 71.4% of the bypasses with competitive or reversed flow at early angiography were occluded 3 years after surgery. Our study revealed that patients with deteriorated grafts were more likely to have ischemic symptoms. There have been no reports investigating the long-term consequences of grafts showing string signs, and further investigation is required.

Mechanism of Graft Deterioration

The mechanism of arterial graft deterioration is suggested by several previous studies. Arterial remodeling is a well-known physiologic adaptation that occurs in response to long-term changes in blood flow to normalize shear stress.[23] For ITA grafts, animal studies have demonstrated that a low-flow condition results in a decreased diameter of the artery accompanied by medial thickening within several months.[24] These findings suggest that the deterioration of an arterial graft may be induced by a low-flow condition, and several clinical studies supported this hypothesis. Akasaka and associates[21] investigated the flow dynamics of the ITA graft showing string sign by use of a Doppler guide wire and demonstrated to and fro signals with systolic reversal and diastolic antegrade flow. Shimizu and coworkers[25] demonstrated decreased graft diameter in a graft with low-flow condition. Tokuda and colleagues[26] reported that a lower mean graft flow and a higher percentage of backward flow as measured by intraoperative transit time flow were independent risk factors for arterial graft deterioration. The results of our study are in agreement with these findings. Graft designs that might decrease the flow at distal anastomoses were revealed to be risk factors for arterial graft deterioration. Mild stenosis of the target coronary artery promotes flow competition. Multiple grafting from a single inflow source may limit the inflow volume and result in decreased graft flow. These findings suggest that a certain amount of graft flow is required to maintain the function of the arterial graft.

Study Limitations

This study has several limitations. First, all data were retrospectively collected, which may have led to information bias. Second, follow-up angiography was performed for only 47.8% of the patients who underwent off-pump CABG during this study period. Angiography was performed according to a protocol and was not symptom directed. We cannot eliminate the possibility that there was a bias in the patient selection. The patient characteristics and the midterm clinical results are compared between the study patients and the excluded patients in Table 3. The differences need to be considered in the interpretation of our

data. Importantly, the survival of the study patients was significantly higher than that of the excluded patients. This finding suggests that the rate of arterial graft deterioration may have been understated in our cohort, which was biased toward healthier patients. Third, the study patients included those with arterial grafts showing focal stenosis (11 instances of distal stenosis in 9 patients) on early angiography. The 11 grafts with focal stenosis seemed to have good graft flow on early angiography. At 1-year angiography, stenosis had disappeared in 5 grafts, and 6 grafts continued to show focal stenosis. None of the grafts with focal stenosis had graft deterioration. Fourth, the use of secondary preventive medication in the study patients was relatively low. Although postoperative medication was not statistically associated with the development of graft deterioration, it is possible that this low use of secondary prevention medication enhanced the development of graft deterioration.

CONCLUSIONS

Arterial graft deterioration 1 year after CABG occurred in 13.8% of all distal anastomoses in arterial grafts. The graft deterioration was closely related to particular graft materials and designs. The incidence of graft deterioration was higher for non-ITA grafts, non-LAD anastomoses, mild (≤75%) stenosis of target coronary arteries, composite grafting, and multiple anastomoses from a single inflow source. The incidence was particularly high when composite or multiple grafting from a single inflow source was performed to a target coronary artery with mild stenosis. When performing multiple arterial grafting, careful attention to the selection of graft material and design is important to gain the full advantage of arterial grafts.

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000 Arterial graft deterioration one year after coronary artery bypass grafting

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We investigated arterial graft deterioration in 243 patients at 1 year after off-pump coronary artery bypass grafting. Multivariate analysis revealed that non–internal thoracic artery graft, mild target stenosis, and multiple grafting were independent predictors of graft deterioration. Arterial graft deterioration was closely related to particular graft materials and designs.

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Increased Graft Occlusion or String Sign in Composite Arterial Grafting for Mildly Stenosed Target Vessels

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Background. Composite grafting is a useful technique that avoids the need for aortic manipulation and enables a wide range of target vessels to be revascularized, effectively using the limited arterial grafts available. However, it has not been clarified whether composite grafting can achieve angiographic outcomes equivalent to those obtained with individual grafting for specific target vessels.

Methods. We retrospectively reviewed 830 distal arterial graft anastomoses in 256 patients who underwent off-pump coronary artery bypass surgery and also underwent 1-year follow-up coronary angiograms. Four hundred and ten anastomoses using a composite grafting technique were compared with 420 anastomoses using individual grafting.

Results. In target vessels with mild stenosis, the incidence of graft occlusion or string sign was significantly higher in composite internal thoracic arteries (ITA) than in individual ITA grafts (composite 20.3% versus individual 7.3%; $p = 0.018$) and showed a higher tendency in composite radial arteries (RA) than in individual RA grafts (59.3% versus 36.4%, $p = 0.09$). In contrast, the incidence was similar between composite and individual ITA grafts (5.7% versus 3.3%, $p = 0.278$) and composite and individual RA grafts (11.5% versus 29.6%, $p = 0.297$) in target vessels with severe stenosis.

Conclusions. The angiographic outcomes of composite grafts were closely related to the severity of stenosis of the target coronary artery. In target vessels with mild stenosis, composite grafting resulted in a higher incidence of graft occlusion or string sign than individual grafting did.

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Modern coronary artery bypass graft surgery (CABG) involves several sophisticated procedures developed to handle particular problems or improve the quality of treatment. The aortic "no-touch" technique is considered effective for reducing stroke risk in patients with the atherosclerotic ascending aorta, and multiple arterial grafting is usually preferred because it provides excellent long-term clinical outcomes. Composite grafting plays a crucial role in these procedures, because it eliminates the need for proximal anastomosis to the ascending aorta and conserves extra lengths of an arterial graft for additional grafting.

Although the prevalence of composite grafting is increasing, there have been few studies to support the feasibility of performing composite grafting for a particular target coronary artery. Several studies reported that the clinical and angiographic results of composite grafting were equivalent to those of individual grafting [1–3]. Conversely, some other studies reported that composite grafting may be susceptible to the detrimental effect of flow competition with native coronary artery when used for a mildly stenosed target vessel [4, 5]. The difference in angiographic outcomes between composite and individual grafting in target vessels with mild stenosis has not been clarified. Hence, the purpose of this study was to compare the angiographic outcomes between composite and individual grafts according to the severity of stenosis of the target coronary artery.

Material and Methods

Study Design

This was a retrospective cohort study to verify the hypothesis that angiographic outcomes of composite grafts

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Table 1. Patient Characteristics

	Total n = 256	Without Composite n = 108	With Composite n = 148	*p* Value*
Age, years	66.3 ± 8.1	66.3 ± 8.1	66.6 ± 7.3	0.776
Male (n)	85.6% (219)	85.2% (92)	85.8% (127)	1.000
Coronary risk factor (n)				
Hypertension	69.9% (179)	69.4% (75)	70.3% (104)	0.891
Diabetes mellitus	41.0% (105)	35.2% (38)	45.3% (67)	0.123
Hyperlipidemia	63.7% (163)	68.5% (74)	60.1% (89)	0.189
Smoking	57.0% (146)	57.4% (62)	56.8% (84)	1.000
Old cerebral infarction (n)	7.4% (19)	4.6% (5)	9.5% (14)	0.227
PVD (n)	6.3% (16)	3.7% (4)	8.1% (12)	0.194
Chronic hemodialysis (n)	3.5% (9)	1.9% (2)	4.7% (7)	0.310

* Comparison between patients with and without composite grafts.

PVD = peripheral vascular disease.

were inferior to those of individual grafts in target vessels with mild stenosis. One-year angiographic outcomes of arterial grafts were reviewed, and incidence of graft occlusion or string sign was compared between composite and individual grafts according to the severity of stenosis of the target coronary artery. Moreover, multivariate analysis was performed to identify the independent predictor of graft occlusion or string sign. The Ethics Committee of Sakakibara Heart Institute approved this study, waived the need for patient consent, and provided approval before the publication of the data.

Study Subjects and Data Collection

Between September 2004 and July 2007, 536 patients underwent isolated CABG in our institute. All patients were scheduled for off-pump CABG. Six patients who were converted to an on-pump CABG were excluded from the study. We routinely performed coronary angiograms 1 year after surgery in patients who have undergone off-pump CABG, regardless of the patient's symptoms. Patients who died, refused angiographic evaluation, were more than 75 years old, or had renal dysfunction (serum creatinine > 1.2 mg/dL) were excluded from the angiographic follow-up. Of the 536 patients, 256 patients (47.8%) underwent 1-year follow-up angiograms and were retrospectively reviewed. Preoperative characteristics of the study patients are shown in Table 1.

In the 256 study patients, there were 1,050 distal anastomoses, an average of 4.1 per patient. Of these, 830 anastomoses were constructed with arterial grafts and 220 were constructed with saphenous vein. All composite grafts were constructed with arterial grafts. Anastomoses constructed with saphenous vein were excluded from the analysis. Among the 830 anastomoses using arterial grafts, 410 anastomoses were constructed with composite graft (composite group) and 420 anastomoses with individual graft (individual group). Both groups included sequential grafting. Graft material and location and stenosis of the target coronary artery are shown in Table 2. Composite grafts were made using an "I" configuration in 37 anastomoses and a "Y" configuration in 373 anastomoses.

One physician initially reviewed all the coronary angiograms, and a consensus was reached after review. For native coronary arteries, mild stenosis was defined as a stenotic lesion producing luminal narrowing of 75% or less, and severe stenosis as narrowing of more than 75%. Distal anastomoses were assessed and classified as patent, focally stenosed, string sign, or occluded. Focally stenosed was defined as a focal stenosis of 90% or greater anywhere within the conduit or at the anastomosis. String sign was defined as luminal narrowing throughout the entire conduit, including stenosis of 90% or more.

Operative Strategy

The surgical procedures and principles of off-pump CABG we used have been previously described [6]. The left-sided coronary arteries were revascularized with arterial grafts in most cases. The left anterior descending artery (LAD) was revascularized exclusively using the

Table 2. Graft Material and Location and Stenosis of Target Coronary Artery

	Composite n = 410	Individual n = 420	*p* Value
Graft material			<0.001
ITA	191 (46.6%)	336 (80.0%)	
RA	211 (51.5%)	49 (11.7%)	
GEA	8 (2.0%)	35 (8.3%)	
Location of target coronary artery			<0.001
LAD	34 (8.3%)	223 (53.1%)	
D	127 (31.0%)	51 (12.1%)	
LCX	234 (57.1%)	108 (25.7%)	
RCA	15 (3.7%)	38 (9.0%)	
Stenosis of target coronary artery			0.021
Mild, ≤ 75%	153 (37.3%)	125 (30.0%)	
Severe, > 75%	257 (62.7%)	295 (70.2%)	

D = diagonal branch; GEA = gastroepiploic artery; ITA = internal thoracic artery; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RA = radial artery; RCA = right coronary artery.

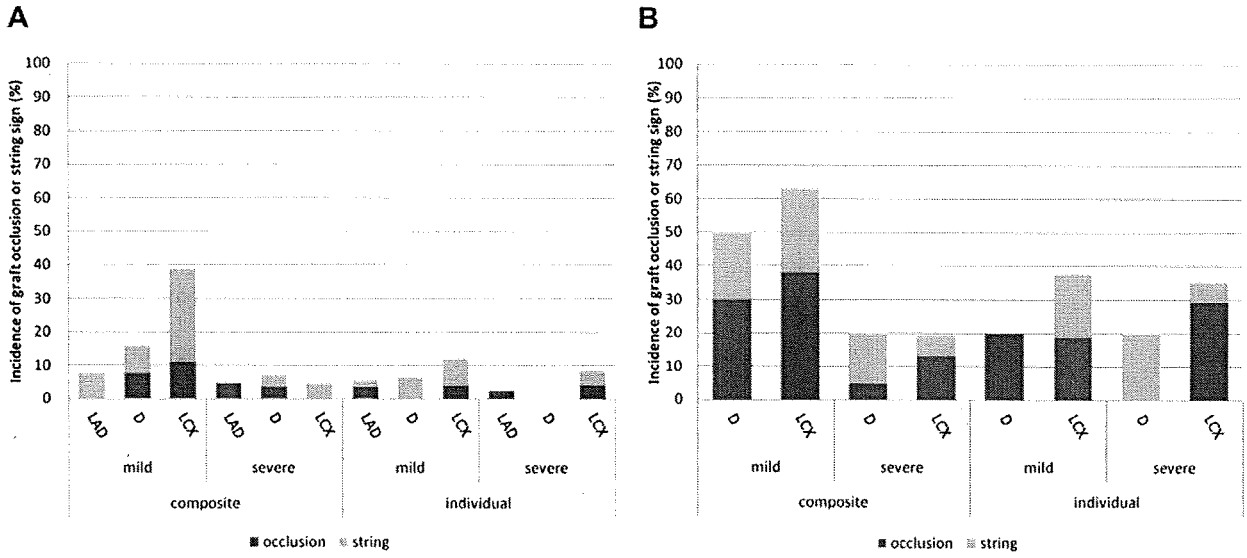

Fig 1. The incidence (%) of graft occlusion (dark gray shaded bar) or string sign (light gray shaded bar) according to graft material or location of target coronary artery. (A) internal thoracic artery (ITA). (B) Radial artery (RA). (D = diagonal artery; LAD = left anterior descending artery; LCX = left circumflex artery.)

internal thoracic artery (ITA), and the left ITA was preferably used. The right ITA was revascularized to the LAD only when the left ITA was required to bypass a remote anastomosis site of the left circumflex artery. The most frequently used arrangement for diagonal artery and left circumflex artery was composite grafting with right ITA and radial artery (RA). In this arrangement, the right ITA was used as an in-situ graft for the diagonal artery, and the RA was anastomosed proximally to the right ITA and distally to the left circumflex artery. The right coronary artery was grafted with saphenous vein or gastroepiploic artery in most cases. Use of the gastroepiploic artery was usually limited to patients with severe stenosis of the right coronary artery.

Statistical Analysis

Categorical variables are reported as percentages. To compare categorical variables, the χ^2 test was used to compare among three groups and the Fisher's exact test was used to compare between two groups. Student's *t* test was used to compare continuous variables. Multivariate analysis was performed to identify independent risk factors for graft occlusion or string sign. A generalized estimating equation method was used to account for within-patient correlation. Covariates included in the generalized estimating equation models were age, sex, hypertension, diabetes mellitus, hyperlipidemia, smoking history, peripheral vascular disease, graft material (ITA or non-ITA), target coronary artery (LAD or non-LAD), stenosis rate of target coronary artery (mild or severe), composite grafting, and sequential grafting. Odds ratios are presented with 95% confidence intervals. Statistical significance was accepted at p less than 0.05. All statistical analyses was performed with SPSS statistical software (SPSS version 17.0; SPSS Japan, Tokyo, Japan).

Results

Incidence of graft occlusion or string sign was compared between composite grafts and individual grafts according to graft material, location of target coronary artery, and stenosis of target coronary artery (Table 3). There were significant differences between composite and individual

Table 3. Incidence of Graft Occlusion or String Sign in Composite and Individual Grafts

	Composite	Individual	*p* Value
Graft material			
ITA	11.0% (21/191)	4.5% (15/336)	0.006
RA	34.6% (73/211)	32.7% (16/49)	0.868
GEA	12.5% (1/8)	22.9% (8/35)	1.000
Location of target coronary artery			
LAD	5.9% (2/34)	3.1% (7/223)	0.339
D	15.0% (19/127)	7.8% (4/51)	0.228
LCX	30.8% (72/234)	17.6% (19/108)	0.052
RCA	13.3% (2/15)	26.3% (10/38)	0.472
Stenosis of target coronary artery			
Mild, ≤ 75%	40.5% (62/153)	13.6% (17/125)	<0.001
Severe, > 75%	12.8% (33/257)	7.8% (23/295)	0.065
≤ 50%	66.7% (14/21)	57.1% (4/7)	0.674
> 50%, ≤ 75%	36.4% (48/132)	11.0% (13/118)	<0.001
> 75%, ≤ 90%	15.1% (23/152)	6.9% (13/188)	0.020
> 90%	9.5% (10/105)	9.3% (10/107)	1.000

D = diagonal branch; GEA = gastroepiploic artery; ITA = internal thoracic artery; LAD = left anterior descending coronary artery; LCX = left circumflex coronary artery; RA = radial artery; RCA = right coronary artery.

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Table 4. Incidence of Graft Occlusion or String Sign According to Severity of Target Coronary Artery

	Composite	Individual	*p* Value
ITA			
Mild, ≤ 75%	20.3% (14/69)	7.3% (7/96)	0.018
Severe, > 75%	5.7% (7/122)	3.3% (8/240)	0.278
≤ 50%	14.3% (1/7)	60.0% (3/5)	0.222
> 50%, ≤ 75%	21.0% (13/62)	4.4% (4/91)	0.003
> 75%, ≤ 90%	5.6% (4/72)	3.2% (5/157)	0.473
> 90%	6.0% (3/50)	3.6% (3/83)	0.672
RA			
Mild, ≤ 75%	59.3% (48/81)	36.4% (8/22)	0.090
Severe, > 75%	11.5% (25/130)	29.6% (8/27)	0.297
≤ 50%	93.0% (13/14)	50.0% (1/2)	0.242
> 50%, ≤ 75%	50.7% (34/67)	35.0% (7/20)	0.308
> 75%, ≤ 90%	23.4% (18/77)	40.0% (6/15)	0.206
> 90%	13.2% (7/53)	16.7% (2/12)	0.667

ITA = internal thoracic artery; RA = radial artery.

grafts in ITA grafts and in the presence of mild stenosis of target coronary artery.

Incidence of graft occlusion or string sign in ITA and RA graft according to severity of target coronary artery is shown in Table 4. In target vessels with severe stenosis, there were no differences in the incidence of graft occlusion or string sign between composite and individual grafts in ITA (composite 5.7% versus individual 3.3%, p = 0.278) and RA (11.5% versus 29.6%, p = 297). But in target vessels with mild stenosis, the incidence of graft occlusion or string sign was significantly higher for composite grafts than for individual grafts in ITA (20.3% versus 7.3%, p = 0.018) and showed a lower tendency in RA (59.3% versus 36.4%, p = 0.09). The incidence of graft occlusion or string sign according to graft material, location and stenosis of the target coronary artery, and graft configuration is shown in Figure 1. The incidences of graft occlusion and string sign were particularly high when composite grafts were used for a mildly stenosed target vessel, irrespective of the graft material or location of the target coronary artery.

The results of multivariate analysis are shown in Table 5. The independent predictors of graft occlusion or string sign in total were non-ITA graft, mild stenosis of the target coronary artery, and peripheral vascular disease. Composite grafting was an independent predictor of graft occlusion or string sign only when grafted to the target vessels with mild stenosis.

Comment

Comparison of Composite and Individual Grafting

The present study revealed that the angiographic outcomes of composite grafts were closely related to the severity of stenosis of the target coronary artery. Several previous studies reported that the patency rate of composite grafts was equal to that of individual grafts [1–3]. However, none of them examined the patency rate in relation to stenosis of the target coronary artery. Suboptimum angiographic results of composited grafting for mildly stenosed target vessels have been reported by several studies. Pevni and colleagues [4] reported that a lower stenosis rate of the target coronary arteries was associated with a higher occlusion rate of composite ITA grafts. Gaudino and associates [5] reported that the threshold of stenosis for graft occlusion in a target coronary artery was higher in composite RA grafts than in individual RA grafts. Nakajima and associates [7] reported that 75% stenosis in the right coronary artery was an independent predictor of competitive flow and graft occlusion. From a practical standpoint, whether there is a difference in angiographic outcomes between composite and individual grafts for particular target vessels has been considered important, but none of these studies compared angiographic outcomes of composite and individual grafting. The present study is the first to demonstrate a higher incidence of graft failure in composite grafting for mildly stenosed target vessels. Moreover, in target vessels with mild stenosis, composite grafting has been shown to be an independent predictor of graft occlusion or string sign. Based on these results, we do not recommend composite grafting in target vessels with mild stenosis.

Mechanism of Graft Failure in Composite Grafts

The precise mechanism of graft failure in composite grafts has not been completely clarified. Arterial grafts are known to narrow diffusely or occlude when they are used in low-flow conditions [6, 8]. The susceptibility of composite grafting to low-flow conditions when used in target vessels with mild stenosis has been suggested by several studies. Studies examining the blood flow of composite grafts reported flow reduction of approximately 20% for composite grafting compared with the

Table 5. Multivariate Analysis of Risk Factors for Graft Occlusion or String Sign

Risk Factors	Odds Ratio	95% CI	*p* Value
Total			
Non-ITA	4.88	2.74–8.69	<0.001
Mild stenosis	3.61	2.29–5.70	<0.001
Composite grafting	1.37	0.70–2.68	0.362
Peripheral vascular disease	2.65	1.21–5.82	0.015
For mildly stenosed target vessels			
Non-ITA	5.80	2.64–12.71	<0.001
Composite grafting	2.73	1.17–6.40	0.021
For severely stenosed target vessels			
Non-ITA	4.26	1.83–9.90	<0.001
Composite grafting	0.72	0.27–1.91	0.509
Peripheral vascular disease	4.55	1.69–12.23	0.003

CI = confidence interval; ITA = internal thoracic artery.

sum of 2 individual grafts [9, 10]. Furthermore, the flow through a composite graft is strongly influenced by native coronary flow. Markwirth and colleagues [11] reported that in composite grafts anastomosed to a patent but stenosed target vessel, the graft flow is lower by 40% than that in grafts anastomosed to occluded target vessels. Nakajima and coworkers [7] reported the incidence of flow competition in composite grafts was as high as 14.6%. These findings suggest that composite grafting may be susceptible to the detrimental effect of flow competition with native coronary artery, resulting in a low-flow condition. This supposition is in agreement with the finding in the present study that mild stenosis of the target coronary artery is related to the incidence of graft occlusion or string sign in composite grafts.

Study Limitations

This study has several limitations. First, all data were retrospectively collected, which may have led to information bias. Second, a follow-up angiogram was performed in only 47.8% of the patients who underwent off-pump CABG during this study period. The angiogram was performed according to a protocol and was not symptom-directed. Third, composite grafting included both I and Y configurations. According to our data, there were no differences in patency rate between these configurations. Fourth, in some graft designs, the number of anastomoses was too small to perform statistical analysis. The number of gastroepiploic arteries was too small to draw any conclusion. The number of individual RA grafts was relatively small, which may have involved a wide variation of the data. Fifth, the graft occlusion and string sign may include intraoperative graft failure, because we did not perform early postoperative angiography in all patients.

In conclusion, the angiographic outcomes of composite grafts were closely related to the severity of stenosis of the target coronary artery. In target vessels with mild stenosis, angiographic outcomes of composite grafts were inferior to those of individual grafts.

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INVITED COMMENTARY

This report by Manabe and colleagues [1] provides a 1-year angiographic follow-up of coronary revascularization using composite grafting, which was compared with revascularization using individual grafting, and composite grafting in target vessels with mild stenosis is not recommended.

Greater freedom from reinterventions and enhanced long-term survival rates have been demonstrated when bilateral internal thoracic arteries (ITAs) are used rather than a single ITA graft in surgical revascularization for multi-vessel coronary disease. The superiority of one method in comparison to another has not been established for bilateral ITA grafting in an individual or composite configuration. A recent study by Kim and colleagues [2] demonstrated that perfusion improvements were similar for both bilateral individual ITA and composite grafts in terms of reversibility scores at 5 years postoperatively.

Revascularization of stenotic coronary lesions that induce myocardial ischemia can improve a patient's functional status and outcome. However, the benefit of revascularization is less clear for mildly stenotic coronary lesions that do not induce myocardial ischemia. Coronary angiography remains the most accurate morphologic assessment of lumen of the coronary arteries. However, angiographically, the degree of stenosis is a poor tool for gauging the functional significance of a specific coronary stenosis. Fractional flow reserve (ie, the ratio of maximal blood flow in a stenotic artery to normal maximal flow) is an index of the physiologic significance of a coronary stenosis, and this can easily be measured during coronary angiography. The combination of anatomic assessment and precise functional information is indispensable in tailoring the revascularization in angiographically dubious stenoses [3].

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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 ≥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 ≥140 mmHg, a diastolic blood pressure ≥90 mmHg or the use of antihypertensive agents. Diabetes was defined as a fasting serum glucose ≥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 ± 0.82 mmol/l (124.9 ± 31.7 mg/dl) in men and 3.49 ± 0.90 mmol/l (134.8 ± 34.9 mg/dl) in women. The mean baseline serum non-HDLC was 3.90 ± 0.89 mmol/l (151.1 ± 34.5 mg/dl) in men and 4.01 ± 1.01 mmol/l (155.2 ± 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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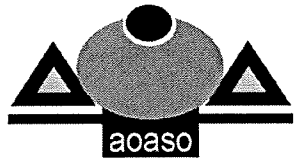

http://www.iotf.org/oonet/aoaso.htm

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).

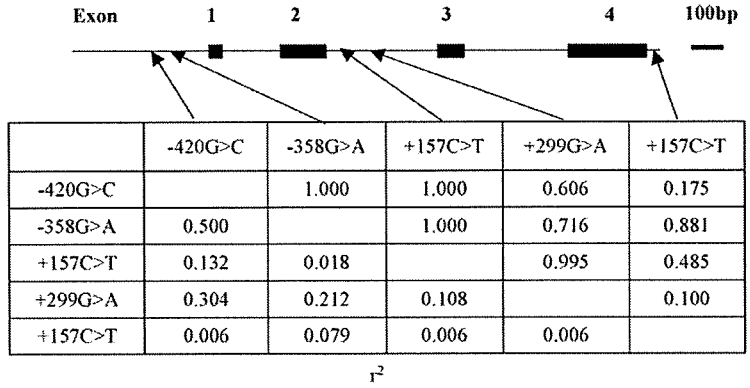

Figure 1 Using five SNPs (−420C > G, −358G > A, +157C > T, +299G > A, +1263G > C) as tags to define haplotypes, we calculated the pair-wise r^2 and D' for each SNP pair and evaluated the linkage disequilibrium pattern for the resistin gene in 2968 subjects.

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.