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

Conclusion

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

Disclosures

None.

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EDITORIAL

Exercise Training in Post-CABG Patients at Low Prognostic Risk

– Beyond Recovery From Surgery –

Yoichi Goto, MD, PhD

Cardiac rehabilitation with exercise training has been shown to improve exercise capacity, coronary risk factors, and health-related quality of life (QOL), to retard the progression of atherosclerosis, and to decrease morbidity and mortality in patients with coronary artery disease (CAD).[1] Based on these lines of evidence, the American College of Cardiology/American Heart Association (ACC/AHA) guidelines recommend cardiac rehabilitation for all eligible patients with CAD, including those after coronary artery bypass grafting (CABG).[2] However, among studies focusing exclusively on a CABG population, the existing evidence of the efficacy of exercise training is limited to improvements in exercise tolerance and psychological sense of well-being.[2,3] In this issue of the Journal, Bilinska et al report the effects of exercise training on hemodynamic and neurohumoral responses to static (handgrip) exercise and on inflammatory markers in patients after CABG.[4] Their study is unique in the following 3 aspects.

Article p 2598

Sympathetic and Metabolic Control of Cardiovascular Response to Exercise

The first of these is that the authors assessed the effects of dynamic exercise training on the response to static exercise. Static exercise is known to elicit a greater increase in systolic blood pressure (BP) than dynamic exercise, but the effects of exercise training on the hemodynamic and neurohumoral responses to static exercise have not been well understood. The finding that the increases in heart rate, systolic BP, total peripheral resistance and plasma norepinephrine concentration during handgrip exercise were attenuated after 6-week exercise training were anticipated, but the finding of the greater increase in the nitric oxide (NO) level in response to handgrip exercise after exercise training is intriguing. Recent studies suggest that not only the sympathetic nerve system but also NO-mediated metabolic regulation significantly contribute to the control of the cardiovascular response to acute exercise or mental stress.[5–7] Therefore, hemodynamic changes, such as increases in BP and vascular resistance during static exercise, are the composite result of interaction between 2 regulatory systems, that is, the sympathoexcitatory α-adrenergic and sympathoinhibitory NO systems.

Sugawara et al reported that, after exercise training, increased NO-mediated vasodilatation is counterbalanced by enhanced α-adrenergic vasoconstriction, resulting in an unchanged basal limb blood flow.[8] Additionally, the Bilinska study demonstrated that both attenuated norepinephrine release and enhanced NO release may be involved in the attenuated increases in systolic BP and peripheral vascular resistance during handgrip exercise after exercise training.[4] These findings may be important for explaining the mechanism of the beneficial cardiovascular effects of exercise training, because there is a view that high levels of baseline sympathetic outflow are not dangerous per se, but that high levels of sympathetic outflow in conjunction with endothelial dysfunction may have synergistic and detrimental effect in terms of cardiovascular risk.[9] If so, a plausible scenario is that the vicious cycle of autonomic dysfunction and endothelial dysfunction can be prevented or ameliorated by regular exercise training.

Effect of Exercise Training on Systemic Inflammation

Secondly, Bilinska et al demonstrate that exercise training results in a significant reduction in inflammatory markers in post-CABG patients. Although previous studies have reported a reduction in inflammatory markers after exercise training in CAD patients,[10,11] this is the first report in post-CABG patients. It is conceivable that, in post-CABG patients, even after active myocardial ischemia is extinguished, the remaining atherosclerotic plaques at the original sites may continue to be a source of chronic inflammation.

The precise mechanisms by which exercise training ameliorates systemic inflammation is unclear, but Handschin and Spiegelman proposed peroxisome proliferative-activated receptor γ (PPARγ) coactivator 1α (PGC-1α) as a key factor in the beneficial effect of exercise.[12] PGC-1α is a critical coordinator of the activation of metabolic genes controlling substrate use and mitochondrial biogenesis, and according to Handschin and Spiegelman, regular exercise induces PGC-1α in skeletal muscles, which in turn suppresses the production of proinflammatory cytokines such as interleukin-6 or tumor-necrosis factor-α in muscles.[12] Conversely, a sedentary lifestyle would decrease PGC-1α expression in skeletal muscles, resulting in elevation of proinflammatory cytokines and hence, chronic systemic inflammation.

Interval vs Endurance Mode of Exercise Training

Thirdly, the study being discussed is unique because the investigators used interval training rather than endurance (continuous) training. Recent studies have demonstrated that high-intensity interval training is more effective than continuous moderate exercise training in enhancing exercise capacity, PGC-1α level, and endothelial function in patients with metabolic syndrome or chronic heart failure.[13,14] If interval training proves to be more effective than endurance training in gaining cardiovascular benefits, the mode of exercise training, and hence, the style of contemporary cardiac rehabilitation, will be greatly changed.

Remaining Issues

The study population was highly selected, young male patients after off-pump CABG with preserved left ventricular function and without myocardial ischemia, uncontrolled coronary risk factors, or comorbidities; that is, the patients were at very low prognostic risk, which means it is not easy to confirm that the observed beneficial effects will translate into meaningful clinical outcome, because the long-term event rate in this population should be very low. In addition, it remains unknown whether the presented findings obtained in a highly selected population can be generalized to real-world patients with multiple risk factors and comorbidities.

Lastly, despite the established and additional potential benefits, the use of outpatient exercise training/cardiac rehabilitation remains very low in Japan.[15] Considering the significant impact of exercise training on both the NO and PGC-1α systems that regulate fundamental cardiovascular pathophysiology, this important therapeutic modality warrants more widespread application.

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ORIGINAL ARTICLE

Cardiac Rehabilitation

Efficacy of Out-Patient Cardiac Rehabilitation in Low Prognostic Risk Patients After Acute Myocardial Infarction in Primary Intervention Era

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Background: The efficacy of out-patient cardiac rehabilitation (OPCR) in patients with a low prognostic risk after acute myocardial infarction (AMI) is unclear in the recent primary intervention era.

Methods and Results: A total of 637 AMI patients who participated in in-hospital cardiac rehabilitation were divided into 2 groups; low prognostic risk group (n=219; age <65 years, successful reperfusion, Killip class I, peak serum creatine kinase <6,000U/L, and left ventricular ejection fraction ≥40%) and non-low prognostic risk group (n=418). The prevalence of coronary risk factors (CRF) was compared between the 2 groups. Then, in the low-risk group, the efficacy of OPCR was compared between active OPCR participants (n=52; ≥20 sessions/3 months) and non-active participants (n=60; <6 sessions/3 months). Compared with the non-low prognostic risk group, the low prognostic risk group had a significantly higher prevalence of current smokers (72% vs. 49%, P<0.05) and patients with multiple CRF (3 or more; 49% vs. 39%, P<0.05). Among the low- risk group, active OPCR participants showed a significantly greater improvement in exercise capacity (peak $\dot{V}O_2$, P<0.05) and maintained a better CRF profile (total cholesterol, triglyceride and blood pressure, all P<0.05) than inactive participants at 3 months.

Conclusions: Low prognostic risk AMI patients have a higher prevalence of multiple CRF than non-low risk patients. Even in this low risk group, active participation in OPCR is associated with improved exercise capacity and better CRF profile. (*Circ J* 2011; **75:** 315–321)

Key Words: Acute myocardial infarction; Cardiac rehabilitation; Coronary risk factors; Exercise capacity; Low prognostic risk

C ardiac rehabilitation (CR) is a comprehensive intervention including medically supervised exercise training, risk factor control, patient education, and psychosocial counseling. CR has been reported to be effective in improving numerous intermediate endpoints, including exertional ischemic symptoms, overall feelings of wellness, exercise tolerance, and coronary risk factors (CRF) in patients with coronary artery disease (CAD).[1-6] In addition, recent meta-analyses of randomized studies on the effects of exercise-based CR in patients with CAD have demonstrated a statistically significant reduction in total and cardiac mortality ranging from 20% to 32%[7-9] in patients undergoing CR compared with those receiving standard medical care. The guidelines from the American College of Cardiology/ American Heart Association and Japanese Circulation Society recommend the use of CR after acute myocardial infarc-

tion (AMI) as Class I.[10-14]

Recently, the widespread use of primary percutaneous coronary interventions (PCI) has enabled early ambulation of patients with AMI by reducing acute phase complications, resulting in minimal physical deconditioning. As a result, many AMI patients leave a hospital early without participating in a recovery phase (phase II) out-patient CR (OPCR) program.[15] However, the necessity and efficacy of OPCR remain unclear in AMI patients who are anticipated to be at low risk in terms of long-term prognosis (ie, non-elderly, successful reperfusion, absence of heart failure, and preserved left ventricular (LV) systolic function).

Accordingly, the purpose of the present study was to clarify the prevalence of CRF and to determine the efficacy of a 3-month OPCR program in such presumably low prognostic risk patients after AMI.
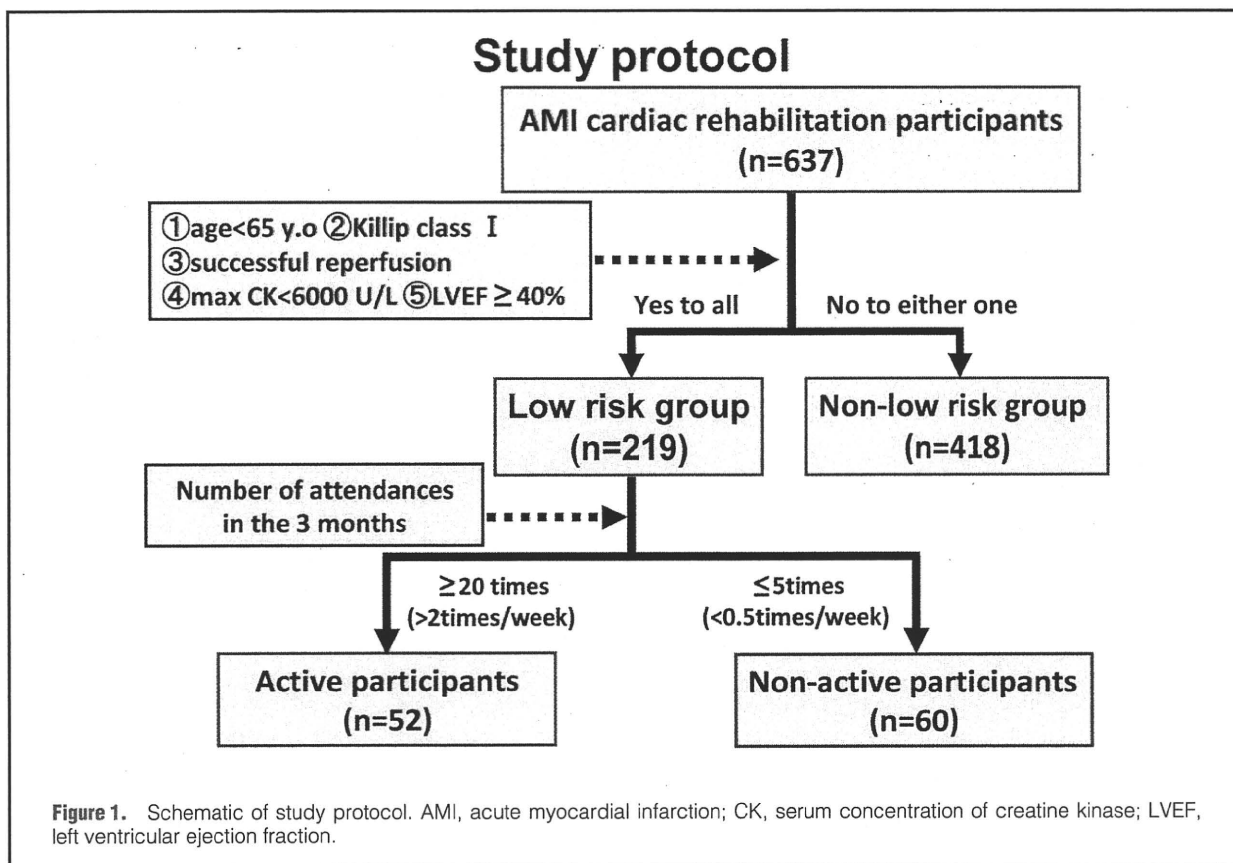
Study protocol

AMI cardiac rehabilitation participants (n=637)

①age<65 y.o ②Killip class I
③successful reperfusion
④max CK<6000 U/L ⑤LVEF ≥ 40%

Yes to all   No to either one

Low risk group (n=219)   **Non-low risk group (n=418)**

Number of attendances in the 3 months

≥20 times
(>2times/week)   ≤5times
(<0.5times/week)

Active participants (n=52)   **Non-active participants (n=60)**

Figure 1. Schematic of study protocol. AMI, acute myocardial infarction; CK, serum concentration of creatine kinase; LVEF, left ventricular ejection fraction.

Methods

Patients

We studied a total of 637 consecutive patients with AMI who participated in a recovery phase CR program and underwent cardiopulmonary exercise testing (CPX) at the beginning and end of a 3-month program in our hospital. The patients were divided into 2 groups: a low prognostic risk group and a non-low prognostic risk group. The low prognostic risk group comprised of 219 patients who fulfilled all of the following criteria indicative of favorable prognosis; age under 65 years, successful reperfusion, Killip class I (an indicator of absence of acute phase heart failure), peak serum creatine kinase (CK) <6,000 U/L, LV ejection fraction (LVEF) ≥40%. The remaining 417 patients who did not fulfill 1 or more of the above 5 criteria were referred to as the non-low prognostic risk group.

As the first step of data analysis, the prevalence each of the CRF (hypertension, hyperlipidemia, diabetes mellitus, obesity and smoking habit) was compared between the low prognostic group and the non-low prognostic group.

As the second step, the efficacy of OPCR in AMI patients at low prognostic risk was examined by comparing the data for exercise capacity and CRF between active participants and non-active participants in the low prognostic risk group. Active participants were defined as patients who attended the OPCR sessions at least 20 times in 3 months (ie, approximately >2times/week), and non-active participants were those who attended OPCR less than 6 times in 3 months (ie, approximately <0.5times/week). There were 52 active participants and 60 non-active participants in the low prognostic group. We did not include the remaining 107 patients with intermediate attendance (patients with 6–19 attendances in 3 months) in the analysis, because the effect of OPCR in this patient group was considered to be modest, if any, and inclusion of this group in the analysis would dilute the measurable efficacy of OPCR. A schematic of the study protocol is provided in **Figure 1**.

CR Program

The CR program began approximately 1 week after AMI and continued after hospital discharge for 3 months. Patients who had angina or evidence of ischemic changes in their electrocardiogram (ECG) at a low level of exercise (walking test), uncontrolled heart failure, and serious arrhythmia were excluded. Program components included supervised exercise sessions (walking, bicycle ergometer and calisthenics) and education, as previously described.[16,17] The exercise intensity was determined individually at 50–60% of heart rate reserve (Karvonen's equation, k=0.5–0.6)[18,19] or a heart rate of anaerobic threshold (AT) level obtained in a maximal symptom-limited CPX testing or at level 12–13 ('a little hard') of the 6–20 scale perceived rating of exercise (original Borg's scale).[20] The exercise program was started with supervised sessions for 2 weeks, followed by home exercise combined with once or twice-a-week supervised sessions for the remaining 10 weeks. Home exercise consisted mainly of brisk walking at a prescribed heart rate for 30 to 60 min, 3–5 times a week.

Patients were encouraged to attend the education classes that were held 4 times a week with lectures on CAD, secondary prevention, diet, smoking cessation, medication, and

Table 1. Comparisons of Clinical Characteristics in the Low and Non-Low Prognostic Risk Groups

	Low-risk group (n=219)	Non-low-risk group (n=418)	P value
Age (years)	55±7	65±9	<0.01
Male (%)	88	83	NS
Killip class ≥II (%)	0	13	<0.01
Peak CK (U/L)	2,458±1,444	3,339±2,639	<0.01
CK ≥6,000 U/L (%)	0	17	<0.001
Unsuccessful reperfusion (%)	0	24	<0.001
LVEF (%)	49.1±6.8	44.4±10.4	<0.01
LVEF <40% (%)	0	34	<0.001
BNP (pg/ml)	75.7±70.9	209.8±202.0	<0.001
HT (%)	57	56	NS
DM/IGT (%)	47	42	NS
HLP (%)	59	49	<0.05
Obesity (%)	28	27	NS
Smoking habit (%)	72	49	<0.001
Coronary risk factors ≥3 (%)	49	39	<0.05

CK, serum concentration of creatine kinase; LVEF, left ventricular ejection fraction; BNP, brain natriuretic peptide; HT, hypertention; DM, diabetes mellitus; IGT, impaired glucose tolerance; HLP, hyperlipidemia.
Values are mean±SD.

Table 2. Baseline Characteristics of Active Participants and Non-Active Participants in the Low-Risk Group

	Active participants (n=52)	Non-active participants (n=60)	P value
Age (years)	57.0±7.3	52.8±7.0	<0.01
Male (%)	83	95	<0.001
Peak CK (U/L)	2,361.1±1,264.2	2,419.5±1,357.1	NS
LVEF (%)	51.4±7.5	47.4±5.7	<0.01
BNP (pg/ml)	83.7±106.0	82.8±74.8	NS
OPCR attendance (times/3 months)	25.5±5.1	1.3±1.7	<0.001
HT (%)	58	52	NS
DM/IGT (%)	44	52	NS
HLP (%)	58	58	NS
Obesity (%)	29	30	NS
Smoking habit (%)	56	75	<0.05
ACE-I/ARB (%)	42	52	NS
β-blocker (%)	19	43	<0.01
Ca channel blocker (%)	40	40	NS
DM medications (%)	8	15	NS
Statin (%)	44	43	NS
Rest HR (/min)	72.6±10.8	71.5±14.9	NS
Rest sBP (mmHg)	123.1±20.2	119.8±21.0	NS
Rest dBP (mmHg)	77.7±11.0	74.7±12.0	NS
Peak WR (W)	132.3±25.2	136.0±31.3	NS
AT (ml·min⁻¹·kg⁻¹)	11.1±2.5	11.6±2.6	NS
Peak V̇O₂ (ml·min⁻¹·kg⁻¹)	23.4±4.2	23.6±5.0	NS
Peak V̇O₂ (%predict)	78.5±14.5	73.7±14.3	NS

Values are mean±SD.
OPCR, outpatient cardiac rehabilitation; ACE-I, angiotensin converting enzyme inhibitor; ARB, angiotensin II receptor blocker; HR, heart rate; sBP, systolic blood pressure; dBP, diastolic blood pressure; WR, work rate; AT, anaerobic threshold; Peak V̇O₂, peak oxygen uptake. Other abbreviations see in Table 1.

physical activities given by physicians, nurses, dieticians, pharmacists and exercise instructors. In addition, all patients received individual counseling on exercise prescription, secondary prevention, and daily life activities by a physician and a nurse at the time of hospital discharge and the end of the 3-month CR program. Patients were scheduled to undergo blood tests at the beginning and the end of the 3-month CR program.
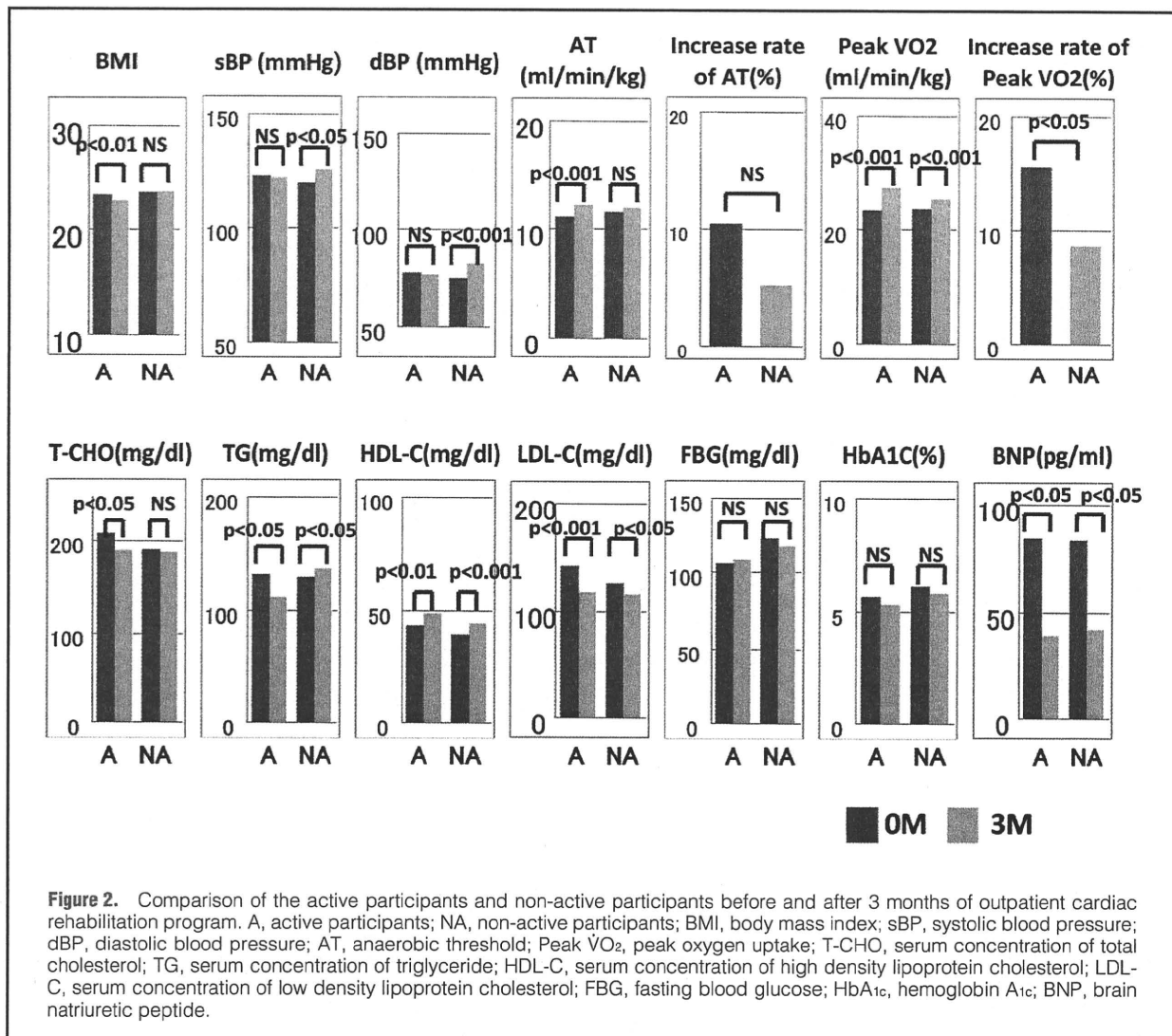
Figure 2. Comparison of the active participants and non-active participants before and after 3 months of outpatient cardiac rehabilitation program. A, active participants; NA, non-active participants; BMI, body mass index; sBP, systolic blood pressure; dBP, diastolic blood pressure; AT, anaerobic threshold; Peak $\dot{V}O_2$, peak oxygen uptake; T-CHO, serum concentration of total cholesterol; TG, serum concentration of triglyceride; HDL-C, serum concentration of high density lipoprotein cholesterol; LDL-C, serum concentration of low density lipoprotein cholesterol; FBG, fasting blood glucose; HbA1c, hemoglobin A1c; BNP, brain natriuretic peptide.

CPX

Patients were scheduled to undergo a symptom-limited CPX at the beginning and the end of the 3-month CR program.[21] After a 2-min rest on the bicycle ergometer in the upright position, the patients started pedaling at an intensity of 0 W for 1 min (warm-up), and then performed an incremental exercise test with a ramp protocol (10 or 15 W/min) until exhaustion. Twelve-lead ECG was continuously monitored and blood pressure (BP) was measured once-a-min with a sphygmomanometer. Expired gas was collected and analyzed continuously with an AE-300S gas analyzer (Minato Co, Osaka, Japan). Peak oxygen uptake (peak $\dot{V}O_2$) was defined as the highest $\dot{V}O_2$ value achieved at peak exercise. Ventilation ($\dot{V}E$) and carbon dioxide output ($\dot{V}CO_2$) were measured and the $\dot{V}O_2$ value at AT or ventilatory threshold was determined as the point at which $\dot{V}CO_2$ increased in a non-linear fashion relative to the rate of $\dot{V}O_2$ (according to the $\dot{V}E/\dot{V}O_2$ time trend, the respiratory exchange ratio flection point, or the V-slope method).[19,22]

Statistical Analysis

Baseline characteristics between the 2 groups were compared using unpaired t-test and chi-square test. Data at baseline and after the 3-month OPCR were compared by paired t-test. A P-value less than 0.05 was considered statistically significant. Data are presented as the mean±standard deviation.

Results

Prevalences of CRF in Low Prognostic Risk Group vs. Non-Low Prognostic Risk Group

Clinical characteristics in the low prognostic risk group and the non-low prognostic risk group are summarized in **Table 1**. Compared with the non-low prognostic risk group, the low prognostic risk group was on average significantly younger, and did not have heart failure on admission or unsuccessful reperfusion, but had lower peak CK and B-type natriuretic peptide (BNP) concentrations and preserved LVEF. Although these findings were anticipated by the definition of the group, they reconfirm that the patients in the low prognostic group were undoubtedly at low prognostic risk. However, when the prevalence of CRF was compared between the 2 groups, the percentage of patients with dyslipidemia, smoking habit and multiple CRF (equal to or more

than 3) was significantly higher in the low prognostic risk group than in the non-low prognostic risk group.

Efficacy of OPCR in Low Prognostic Risk Group: Comparison Between Active and Non-Active Participants

Baseline characteristics in active participants and non-active participants in the low prognostic risk group are summarized in **Table 2**. Although active participants were significantly older than the non-active participants, they were both non-elderly (less than 65 years old). Peak CK was low and LVEF was relatively preserved in both groups. These findings reconfirm that both active and non-active participants are apparently at low prognostic risk. Although there were minor differences in the prevalence of male patients, smokers and β-blocker use, there were no significant differences in exercise capacities at baseline between the 2 groups.

During the 3-month OPCR period, only a few patients experienced changes in medication; statins were introduced in 3 patients (5.8%) in the active participants and 2 patients (3.3%) in the non-active participants, and diabetic medications were started in 2 patients (3.3%) in the non-active participants. Thus, the baseline clinical characteristics of active and non-active participants were almost equivalent, except for the frequency of OPCR attendance.

Figure 2 depicts comparisons of parameters before and after the 3-month OPCR between active and non-active participants in the low prognostic risk group. After the 3-month OPCR, only active participants, and not the non-active participants, showed significant improvements in body mass index (BMI; 23.3±2.5 to 22.9±2.5, $P<0.01$), AT (11.1±2.5 to 12.7±2.5 ml·min^{-1}·kg^{-1}, $P<0.001$), total cholesterol (208.4±33.7 to 188.8±26.4 mg/dl, $P<0.05$), and triglyceride (130.0±77.4 to 111.0±63.7 mg/dl, $P<0.05$). In addition, while peak $\dot{V}O_2$ increased in both groups (active participants 23.4±4.2 to 27.3±5.0 ml·min^{-1}·kg^{-1}, $P<0.001$; non-active participants 23.7±5.0 to 25.3±5.3 ml·min^{-1}·kg^{-1}, $P<0.001$), the magnitude of the increase was significantly greater in the active participants (15.6% vs. 8.6%, $P<0.05$). In contrast, only non-active participants showed significant worsening in systolic and diastolic BP (systolic BP: from 119.8±21.0 to 126.1±20.4 mmHg, $P<0.05$, diastolic BP: from 74.7±12.0 to 82.4±11.8 mmHg, $P<0.001$) and triglyceride (128.0±57.1 to 135.3±63.9 mg/dl, $P<0.05$). The following parameters showed significant improvements both in the active and non-active participants; high density lipoprotein cholesterol (HDL-C: 43.6±14.1 to 49.0±12.2 mg/dl, $P<0.01$; 39.7±11.0 to 44.8±11.6 mg/dl, $P<0.001$), low density lipoprotein cholesterol (LDL-C: 140.1±31.9 to 117.6±25.9 mg/dl, $P<0.001$; 124.8±31.2 to 115.3±19.7 mg/dl, $P<0.01$), and BNP (83.7±106.0 to 39.7±44.8 pg/ml, $P<0.05$; 82.9±74.8 to 42.4±51.7 pg/ml, $P<0.05$).

Discussion

The major findings of the present study are that the low prognostic risk AMI patients had a higher prevalence of smoking habit, dyslipidemia and multiple CRF than the non-low prognostic risk patients, and that in the low prognostic risk group, active participation in OPCR was associated with better CRF profile (ie, BP, dyslipidemia, and obesity) and exercise capacity. These findings suggest that, by actively participating in OPCR after AMI, even the low prognostic risk patients might gain clinical benefits such as better CRF modification and physical functioning.

Previous Studies

Various guidelines for management of post-AMI (or established CAD) patients recommend aggressive modifications of CRF for secondary prevention,[10,12,13] and adherence to these recommendations and/or reduction of CRF have been shown to improve long-term prognosis.[23–26] In contrast, Thrombolysis In Myocardial Infarction (TIMI) risk score[27] and Controlled Abciximab and Device Investigation to Lower Late Angioplasty Complications (CADILLAC) risk score[28] have demonstrated that 1-year mortality is very low in AMI patients with age <65 years, successful reperfusion, absence of acute phase heart failure, and preserved LV function, which are compatible with the patient characteristics of the low prognostic risk group in the present study. However, little is known about the prevalence of CRF or clinical significance of accumulation of multiple CRF in such low prognostic risk patients. In relation to this, it is of note that, Lloyd-Jones and colleagues demonstrated that young subjects with accumulated CRF, despite low short-term risk, have a higher 'lifetime risks for CAD' and greater progression of subclinical coronary atherosclerosis compared with those at low lifetime risk.[29,30] These data suggest that apparently low prognostic risk patients stratified by TIMI or CADILLAC risk score are likely to have superb short-term (1 year) prognosis, but not necessarily favorable long-term or lifetime prognosis.

Present Study

The present study has explicitly demonstrated that the low prognostic risk patients actually have higher prevalence of multiple CRF than the non-low prognostic risk patients. Although the finding that younger AMI patients have higher prevalences of smoking and hyperlipidemia than elderly patients is in accordance with previous studies,[31] there has been no report demonstrating higher prevalence of multiple CRF in low prognostic risk AMI patients with successful reperfusion and preserved LVEF. According to TIMI risk score[27] or CADILLAC risk score,[28] this finding might appear confusing or counterintuitive. However, from the viewpoint of lifetime CAD risk,[29,30] this finding might have a significant impact on the long-term prognosis of apparently low prognostic risk AMI patients.

The second major finding in the current study is that active participation in OPCR improved CRF (BP, dyslipidemia, and obesity) and exercise capacity even in the low prognostic risk group. There have been no studies that reported the effect of OPCR in the low prognostic risk AMI patients. Taylor et al[9] reported in a meta-analysis of randomized controlled trials that the effect of OPCR on total mortality did not differ between studies before and after year 1995 (odds ratio 0.84 before 1995 vs. 0.62 after 1995, NS), but they did not assess the effect of OPCR on the low prognostic risk patients after successful reperfusion. Witt et al recently reported that participation in OPCR after AMI was associated with improved survival and reduced recurrent myocardial infarction (MI) at 3 years, but the rate of reperfusion was only 33% in their patients.[32] Squires et al reported that a 3-year coronary disease management program in OPCR for CAD patients was effective in achieving the secondary prevention goals, but their assessment did not target the low prognostic risk patients.[33] Thus, the present study has demonstrated for the first time the favorable effects of OPCR on CRF and exercise capacity in the low prognostic AMI patients.

Clinical Implications

It remains unknown whether the improvements in CRF profiles and exercise capacity achieved by active participation in OPCR can lead to an improved long term prognosis in the low prognostic risk AMI patients. However, Tani et al reported that successful life style modification with exercise, body weight reduction and smoking cessation for 6 months was associated with coronary plaque volume regression in low prognostic risk CAD patients.[34] Belardinelli et al reported in the ETICA (Exercise Training Intervention after Coronary Angioplasty) trial that a 6-month OPCR for the relatively low risk CAD patients after successful PCI (49% having AMI) reduced cardiac events and hospital re-admission during the follow-up period (33±7 months).[35] In addition, because the magnitude of the improvement in endothelial function afforded by OPCR does not correlate with the improvements in CRF,[36] the general consensus at present is that the favorable effect of OPCR on the long-term prognosis is mediated by a direct anti-atherosclerosis effect of exercise training rather than by improvements in CRF.[4] Therefore, further study is necessary to determine the long term effect of OPCR in AMI patients with low prognostic risk.

In the present study, significant differences were found between active and inactive OPCR participants in BMI, total cholesterol, triglyceride and BP, but not in LDL-C or glucose tolerance. One might argue that the prognostic impacts of BMI, total cholesterol, triglyceride and BP might be less powerful compared with those of LDL-C and diabetes. However, Nakatani et al reported that the metabolic syndrome, diagnosed from the combination of BMI, HDL-C, triglyceride, BP, and fasting blood glucose, was an independent predictor of subsequent combined cardiac events of cardiac death and non-fatal MI in Japanese patients after AMI.[37] Therefore, it is plausible that the improvements in BMI, triglyceride and BP observed in the present study might contribute to the improvement in the long-term prognosis in Japanese AMI patients.

Future Direction

In the present study, the rate of active OPCR participation was only 24% (52/219 patients) in the low prognostic risk group. To reduce lifetime CAD risk in these low prognostic risk AMI patients, a substantial increase in participation rate in OPCR is necessary. However, according to a recent nation-wide survey in 526 Japanese Circulation Society authorized cardiology training hospitals,[15] the implementation rate was 92% for emergency PCI, but only 9% for OPCR. In addition, Ades et al reported that, by multivariate analysis, the strength of the physician's recommendation for participation was the most powerful predictor of OPCR participation.[38] Thus, to increase the participation rate in OPCR, it is critically important to greatly increase the number of CR facilities and to enhance physicians' understanding of the benefits of OPCR after AMI.

Study Limitations

First, this study was a retrospective analysis and the number of patients was relatively small. The more active patients would be expected to participate in OPCR and this might have introduced a selection bias.

Second, the low prognostic risk group is anticipated to be at low risk in terms of short-term prognosis[27,28] and hence, whether improvements in CRF profile in such low prognostic risk patients are associated with actual improvements in outcome is uncertain. A longer follow-up in a larger number of patients is necessary to increase the statistical power to demonstrate the beneficial effect of OPCR on the long-term prognosis.

Conclusions

The low prognostic risk AMI patients have a higher prevalence of multiple CRF than the non-low risk patients. Active participation in OPCR program is associated with improved exercise capacity and CRF profile in such low prognostic risk patients. OPCR program can be effective in achieving secondary prevention goals even in the low prognostic risk AMI patients.

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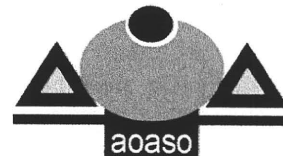
ORIGINAL ARTICLE

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

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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 downregulated 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 $\geq$85 cm for men and $\geq$90 cm for women) plus any two of the following three factors: dyslipidemia (triglycerides $\geq$1.69 mmol/l (150 mg/dl) and/or high-density lipoprotein (HDL) cholesterol $\leq$1.03 mmol/l (40 mg/dl) or lipid-lowering therapy), hypertension (systolic BP $\geq$130 and/or diastolic BP $\geq$85 mmHg, or antihypertensive therapy), and fasting plasma glucose $\geq$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 ($n = 2968$).

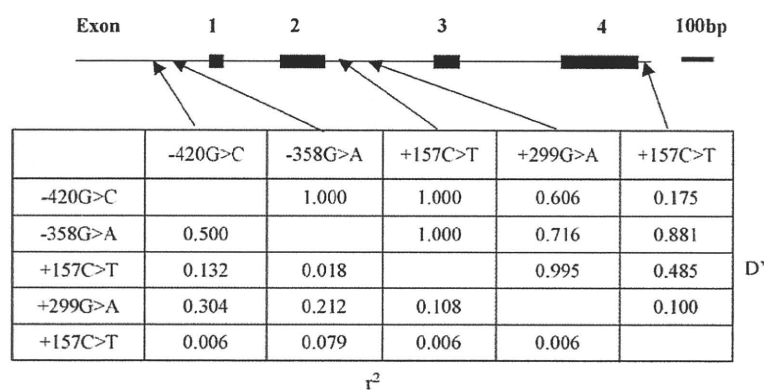|	−420G>C	−358G>A	+157C>T	+299G>A	+157C>T
−420G>C		1.000	1.000	0.606	0.175
−358G>A	0.500		1.000	0.716	0.881
+157C>T	0.132	0.018		0.995	0.485
+299G>A	0.304	0.212	0.108		0.100
+157C>T	0.006	0.079	0.006	0.006	

r^2

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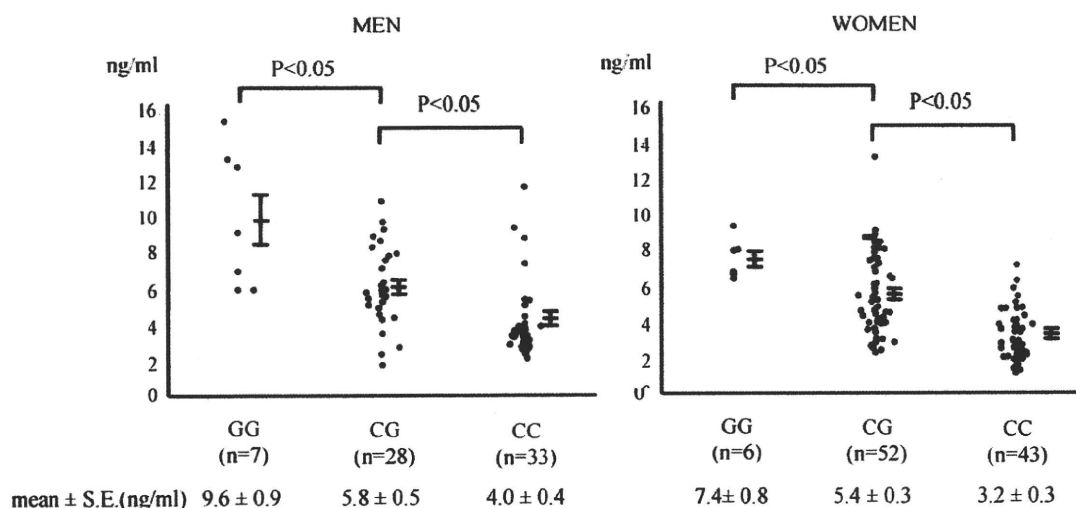
Figure 2   Plasma resistin concentration and resistin −420C > G genotype in Japanese men and women.

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.

Acknowledgments

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