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Association between functional variants of the *ICAM1* and *CRP* genes and metabolic syndrome in Taiwanese subjects

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Abstract

Although inflammation has been shown to play an important role in metabolic syndrome (MetS), the association between inflammatory marker gene polymorphisms and the risk of MetS has not been fully elucidated. This study was initiated to investigate the association between functional variants of inflammatory marker genes and the risk of MetS in Taiwanese adults. The sample population comprised 615 unrelated subjects, of which 22% had MetS. The single nucleotide polymorphisms rs5491 on the intercellular adhesive molecule 1 (*ICAM1*) gene and rs3091244 on C-reactive protein (*CRP*) were genotyped. The *ICAM1* rs5491 polymorphism was significantly associated with the level of soluble intercellular adhesive molecule 1 ($P < .001$). Both the *ICAM1* rs5491 and the *CRP* rs3091244 were shown to have significant association with MetS after adjustment for age, sex, smoking, and body mass index, but not after adjustment for levels of the respective serum marker. Independent associations between the combined *ICAM1-CRP* (rs5491 and rs3091244) genotypes and MetS were found by multivariate analysis ($P = .005$). In subgroup analysis, association of combined genotypes with insulin resistance and MetS mainly occurred in subjects with central obesity. In conclusion, inflammatory marker gene polymorphisms play an important role in modulating the risk of insulin resistance and MetS for subjects with central obesity. These findings will contribute toward a better understanding of the mechanism of association between inflammatory markers and the risk of developing atherosclerotic disease.

1. Introduction

Metabolic syndrome (MetS) is a complex syndrome with clustering of multiple cardiovascular risk factors including central obesity, atherogenic dyslipidemia, hyperglycemia, and hypertension. Although the clinical definition of MetS may vary between defining agencies, the existence of it is indisputable; and understanding its etiology is of high importance [1]. Central obesity has been suggested to be the cardinal feature of MetS, with insulin resistance as a key link between abdominal fat and the risk of developing atherosclerotic cardiovascular disease [2,3]. A newer model for MetS pathogenesis revealed that MetS is associated with dysregulated adipose tissue, in part a consequence of adipose cell enlargement and the associated macrophage infiltration, followed by overexpression of inflammatory cytokines [1,4]. This proinflammatory state leads to local insulin resistance in adipose tissue, liver, and skeletal muscle by impairing insulin signal transduction. The insulin resistance further enhances the proinflammatory state through a feed-forward mechanism that suppresses the antilipolytic and anti-inflammatory effects of insulin [1].

Although inflammation was shown to play an important role in MetS, the association between inflammatory marker

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gene polymorphisms and the risk of MetS has not been fully elucidated. Ridker et al [5] noted a gradual increase in median C-reactive protein (CRP) levels in relation to the number of components of MetS in an 8-year follow-up study of 114 719 initially healthy American women. Our data also revealed that soluble intercellular adhesive molecule 1 (sICAM1) levels were associated with insulin resistance and MetS in a Taiwanese population [6]. Both CRP and sICAM1 are important inflammatory markers. Szalai et al [7] reported that CRP gene promoter harboring the T or A alleles of single nucleotide polymorphism (SNP) rs3091244 (located in a consensus E-box element) is associated with higher promoter activity. Single nucleotide polymorphism rs5491, a missense variant also called *ICAM1*^{Kilifi}, is located in domain 1 near the NH2 terminus of the ICAM1 protein, a region critical for binding with the malarial organism *Plasmodium falciparum*, human rhinovirus, lymphocyte function associated–1, and fibrinogen [8-14]. In vitro studies demonstrated that *ICAM1*^{Kilifi} has an altered ability to bind to *P falciparum*, reduced affinity for lymphocyte function associated–1, and no apparent affinity for fibrinogen [15,16]. The SNP rs3091244 has been shown to be associated with CRP levels [7,17-21], whereas *ICAM1*^{Kilifi} has been shown to be associated with cerebral malaria [15,22]. For further elucidating the relationship between inflammatory gene polymorphisms and the risk of MetS, functional variants on the *CRP* and *ICAM1* genes were analyzed.

2. Subjects and methods

2.1. Subjects

A total of 615 Han Chinese subjects (326 men, 289 women), who had no known history of major systemic inflammation or infection diseases, were recruited during routine health examinations. Exclusion criteria included a history of myocardial infarction, stroke or transient ischemic attack, cancer, and current renal or liver disease. The subjects underwent physical examination that involved measurement of height, weight, waist and hip circumference, and blood pressure (BP) in the sitting position after 15 minutes of rest. Fasting blood samples were obtained from each subject. Table 1 presents the baseline characteristics for the study population. *Diabetes mellitus* was defined as blood glucose levels before a meal of at least 126 mg/dL or the regular use of medication for diabetes mellitus. *Central obesity* was defined as waist circumference greater than 90 cm for men and greater than 80 cm for women (modified criteria for Asians) [23]. *Current smoker* was defined as smoking at least 1 cigarette per day at the time of survey. All subjects provided informed consent. The study protocol

Table 1
Demographic variables and laboratory data for the 615 subjects

	Total (N = 615)	Non-MetS (n = 480)	MetS (n = 135)
Age, y	46 ± 10	45 ± 10	49 ± 10*
Male/female, %	53/47	52.5/47.5	54.8/45.2
Waist circumference, cm	85.20 ± 9.59	83.20 ± 8.96	92.32 ± 8.29*
WHR	0.87 ± 0.06	0.86 ± 0.06	0.91 ± 0.06*
SBP, mm Hg	115.12 ± 17.62	112.30 ± 16.39	125.14 ± 18.25*
DBP, mm Hg	75.97 ± 10.59	74.51 ± 10.29	81.17 ± 10.00*
BMI, kg/m^2	24.33 ± 3.47	23.70 ± 3.25	26.59 ± 3.29*
Total cholesterol, mg/dL	198.17 ± 36.59	196.11 ± 36.29	205.50 ± 36.83*
HDL-C, mg/dL	55.12 ± 14.26	57.85 ± 14.03	45.41 ± 10.35*
LDL-C, mg/dL	115.66 ± 32.86	115.29 ± 33.12	116.98 ± 31.98
Triglycerides, mg/dL	142.21 ± 117.71	117.20 ± 73.67	231.13 ± 184.07*
Fasting plasma glucose, mg/dL	97.12 ± 23.82	92.94 ± 14.26	112.01 ± 39.84*
Smokers, %	24.9	22.3	34.1*
CRP rs3091244 genotypes*			
AA	15 (2.5%)	13 (2.7%)	2 (1.5%)
AC	166 (27.3%)	119 (25.1%)	47 (35.1%)
AT	8 (1.3%)	7 (1.5%)	1 (0.7%)
CT	38 (6.2%)	29 (6.1%)	9 (6.7%)
TT	2 (0.3%)	0 (0%)	2 (1.5%)
CC	380(62.4%)	307 (64.6%)	73 (54.5%)
CRP rs3091244 allele frequency			
C/A/T, %	79.1/16.7/4.1	80.2/16.0/3.8	75.4/19.4/5.2
ICAM1 rs5491 genotypes			
TT	559 (92.2%)	429 (90.9%)	130 (97.0%)
TA	45(7.4%)	41 (8.7%)	4 (3.0%)
AA	2(0.3%)	2 (0.4%)	0 (0%)
ICAM1 rs5491 allele frequency*			
T/A, %	96/4.0	95.2/4.8	98.5/1.5

* $P < .05$.

was approved by the ethics committee of Chang Gung Memorial Hospital.

2.2. Blood chemistry

Total cholesterol and triglyceride concentrations were measured by automatic enzymatic colorimetry. Levels of high-density lipoprotein cholesterol (HDL-C) were measured enzymatically after phosphotungsten/magnesium precipitation. Low-density lipoprotein cholesterol (LDL-C) levels were calculated using the Friedewald formula. Glucose was enzymatically determined by the hexokinase method. Serum insulin levels were measured using an immunoradiometric assay (Bio-source, Nivelles, Belgium). The homeostasis model assessment of insulin resistance (HOMA-IR) index was calculated with the following formula: fasting serum insulin concentration (in microunits per milliliter) × fasting plasma glucose concentration (in millimoles per liter)/22.5. Insulin resistance was recognized when the HOMA-IR index reached the upper quartile. Levels of high-sensitivity CRP (hsCRP) and sICAM1 were measured using sandwich enzyme-linked immunosorbent assays that were developed in-house but, when comprehensively tested, showed good to excellent correlation with commercial enzyme-linked immunosorbent assay kits [24,25].

2.3. Definitions of MetS

Metabolic syndrome characteristics were based on the recent update of the third report of the National Cholesterol Education Program's Adult Treatment Panel III (ATP-III) criteria [26]. According to the ATP-III criteria, subjects with 3 or more of the following attributes are typically defined as having MetS: (1) BP of at least 130/85 mm Hg and/or taking medication for hypertension; (2) triglycerides of at least 150 mg/dL; (3) HDL-C less than 40 mg/dL for men and less than 50 mg/dL for women; (4) fasting plasma glucose of at least 100 mg/dL and/or taking medication for diabetes mellitus; and, (5) waist circumference greater than 90 cm for men and greater than 80 cm for women (modified criteria for Asians) [23].

2.4. Genomic DNA extraction and genotyping of the ICAM1 rs5491 (T>A) polymorphism

Genomic DNA was extracted from peripheral blood leukocytes with a standard method using proteinase K digestion of the nuclei. Phenol and chloroform extractions were followed by DNA precipitation using isopropanol. The *ICAM1* gene rs5491 polymorphism was genotyped by polymerase chain reaction (PCR) and restriction fragment length polymorphism analysis using the primer pair (forward) 5′-AGGCTCCGTGCTGGTGACATTCA-3′ and (reverse) 5′-CTCCGGGCTCAGTTACTCACAGT-3′, and a *Nla*III restriction enzyme.

2.5. Genotyping of the CRP rs3091244 (−286C>A>T) polymorphism

CRP rs3091244 polymorphism was genotyped by PCR and restriction fragment length polymorphism analysis using the primer pair (forward) 5′-ATTTCCCAGTCTGTAAA-TAAGCAAA-3′ and (reverse) 5′-AATGGGAAATGG-TAACATATTAATC-3′. The reverse primer was modified (underlined) to create a *Taq*I endonuclease cleavage site to detect the C allele. The same PCR products were also digested with a *Bfa*I restriction enzyme to differentiate between the A and T alleles [21].

2.6. Statistical analysis

The χ^2 test was used to examine differences among categorical variables. Clinical characteristics of continuous variables were expressed as means ± SD and tested using a 2-sample *t* test or analysis of variance. A general linear model was applied to analyze individual phenotypic components of MetS with respect to predictors of investigated genotypes and confounders. Multiple logistic regression analysis, adjusted for the presence of established risk factors, evaluated the independent effect of genotypes on risk for MetS. Triglyceride and CRP concentrations were logarithmically transformed before statistical analysis to adhere to a normality assumption. A value of $P < .05$ using 2-sided tests was considered statistically significant. Analysis of deviation from the Hardy-Weinberg equilibrium was performed using the SNPStats software package (available from http://bioinfo.iconcologia.net/SNPstats).

3. Results

3.1. Baseline characteristics and genotype frequencies of the CRP and ICAM1 gene polymorphisms in the study population

Table 1 lists the baseline characteristics of the study subjects. Metabolic syndrome prevalence was 22% (135/615). The percentage of male and female subjects with MetS was not significantly different. Age and the prevalence of smoking were greater in subjects with MetS than in subjects without MetS. The waist circumference, waist to hip ratio (WHR), body mass index (BMI), systolic and diastolic blood pressure (SBP and DBP, respectively), serum levels of total cholesterol and triglycerides, and the fasting plasma glucose level were also greater in subjects with MetS, whereas the serum levels of HDL-C were lower in subjects with MetS than in subjects without MetS. Among the individual components in MetS subjects, central obesity was the most common abnormality (81.5%, 110/135), followed by hypertriglyceridemia (72.6%, 98/135), high fasting plasma glucose (58.5%, 79/135), low HDL-C levels (56.3%, 76/135), and high BP (56.3%, 76/135). The genotype and allele frequencies of *ICAM1* rs5491 and *CRP* rs3091244

Table 2
The sICAM1 and hsCRP levels by respective *ICAM1* genotypes and *CRP* genotypes

ICAM1 rs5491 genotypes	sICAM1 levels (ng/mL)	*P*1 value	*P*2 value
TT (n = 554)	250.0 ± 110.1	<.001	<.001
TA (n = 44)	140.0 ± 51.1		
AA (n = 2)	107.8 ± 41.9		
CRP rs3091244	hsCRP levels[a] (mg/L)		
AA (n = 15)	0.93 (0.59-1.79)	<.001	.001
AC (n = 166)	0.78 (0.36-2.14)		
AT (n = 8)	0.88 (0.61-1.31)		
CT (n = 38)	0.76 (0.37-2.98)		
TT (n = 2)	1.01 (0.52-1.50)		
CC (n = 380)	0.52 (0.22-1.11)		
AA + AC + AT (n = 189)	0.82 (0.38-2.06)	<.001	<.001
CT + TT (n = 40)	0.76 (0.38-2.91)		
CC (n = 380)	0.52 (0.22-1.11)		

*P*1: unadjusted. *P*2: multiple linear regression adjusted for age, sex, BMI, SBP, DBP, smoking, diabetes mellitus, total cholesterol, triglyceride, HDL-C, LDL-C, and fasting plasma glucose.

[a] High-sensitivity CRP levels are presented with median and (interquartile range).

polymorphisms in the study samples are shown in Table 1 according to whether the subject has MetS or not.

3.2. Association between ICAM1 gene polymorphisms and sICAM1 levels

No significant deviation from the Hardy-Weinberg equilibrium was detected for rs5491 polymorphisms (P = .25). Our results indicated a strong association between rs5491 T allele and elevated sICAM1 levels (P < .001, Table 2). TA heterozygotes had higher sICAM1 levels (140.0 ± 51.1 ng/mL) than AA homozygotous subjects (107.8 ± 41.9 ng/mL), and the TT homozygotes had the highest sICAM1 levels (250.0 ± 110.1 ng/mL). A multiple linear regression analysis demonstrated that the *ICAM1* rs5491 remained an independent predictor of sICAM-1 levels when age, sex, BMI, SBP, DBP, smoking, diabetes mellitus, total cholesterol, triglyceride, HDL-C, LDL-C, and fasting plasma glucose were taken into account (P < .001, Table 2).

3.3. Association of ICAM1 rs5491 and CRP rs3091244 polymorphisms with components of MetS and Insulin resistance

Table 3 shows the association of the *ICAM1* rs5491 and *CRP* rs3091244 polymorphisms with individual metabolic abnormalities. Neither polymorphism was found to be associated with any component of MetS and insulin resistance.

3.4. Association of ICAM1 rs5491 and CRP rs3091244 polymorphisms with MetS (ATP-III, Asian criteria)

Significantly lower sICAM1 levels were observed in subjects carrying the *ICAM1* rs5491 A allele (AA + AT genotypes) than in noncarriers (TT genotype) after adjustment for sex, age, smoking, and BMI (P < .001, Table 4). The probability of MetS was higher in subjects with the *ICAM1* rs5491 TT genotype carrier than in noncarriers (odds ratio [OR] = 4.70; 95% confidence interval [CI], 1.52-14.49; P = .007; Table 4) after adjustment for sex, age, smoking, and BMI. The *ICAM1* rs5491 TT genotype remained significantly associated with increased risk of MetS when the sICAM1 level was added to the model (Table 4, P = .027). However, this significance became insignificant after stringent Bonferroni correction for multiple tests (2 SNPs >0.025). As previously reported [21], minor alleles of SNP rs3091244 (alleles A and T) were associated with higher CRP levels in our study population (Table 2). The rs3091244 non-CC genotype carriers were, though marginally, significantly associated with an increased risk of MetS (OR = 1.62; 95% CI, 1.05-2.49; P = .029; Table 4) after adjustment for age, sex, smoking, and BMI. The addition of the hsCRP levels to the model resulted in a loss of statistical significance of the relationship between the rs3091244 non-CC genotype and MetS (P = .207, Table 4).

3.5. Association of combined ICAM1 rs5491 and CRP rs3091244 polymorphisms with MetS

We also combined 2 genotypes to study the associations with MetS. As shown in Table 5, subjects with combined *CRP* rs3091244 non-CC genotype and *ICAM1* rs5491 TT genotype were associated with the highest hsCRP and

Table 3
Associations of *ICAM1* rs5491 and *CRP* rs3091244 polymorphisms with components of MetS and insulin resistance

Genotype	*ICAM1* rs5491			*CRP* rs3091244		
	TT (559)	AT + AA (47)	*P* value	CC (380)	Non-CC (229)	*P* value
Waist circumference (cm)	85.3 ± 9.7	85.3 ± 7.6	.979	85.1 ± 9.5	85.6 ± 9.8	.487
WHR	0.87 ± 0.06	0.87 ± 0.05	.630	0.87 ± 0.06	0.87 ± 0.06	.257
HDL-C (mg/dL)	55.15 ± 14.23	54.11 ± 15.30	.632	55.25 ± 14.59	54.72 ± 13.82	.654
Triglyceride (mg/dL)	143.43 ± 121.07	124.06 ± 62.23	.279	136.64 ± 103.10	150.03 ± 137.79	.173
SBP (mm Hg)	115.3 ± 17.8	112.3 ± 14.9	.271	115.4 ± 17.2	114.6 ± 18.3	.577
DBP (mm Hg)	76.0 ± 10.6	74.3 ± 9.7	.280	76.2 ± 10.5	75.5 ± 10.6	.416
Fasting plasma glucose (mg/dL)	97.41 ± 24.71	94.38 ± 11.82	.406	97.28 ± 24.19	96.97 ± 23.50	.877
Fasting serum insulin (μIU/mL)	9.5 ± 5.4	8.4 ± 3.1	.177	9.2 ± 4.8	9.7 ± 6.1	.307
HOMA-IR index	2.3 ± 1.6	2.0 ± 0.8	.140	2.2 ± 1.3	2.4 ± 1.9	.174

Table 4
Association of *ICAM1* rs5491 and *CRP* rs3091244 polymorphisms with MetS (ATP-III, Asian criteria)

ICAM1 rs5491 genotype	TT (559)	AT + AA (47)	*P*1 value	*P*2 value	*P*3 value
sICAM1 levels (ng/mL)	250.0±110.1	138.5±50.8	<.001	<.001	
MetS, yes/no (%)	130/429 (23.3%)	4/43 (8.5%)	.019	.007	.027
CRP rs3091244 genotype	CC (380)	Non-CC (229)			
CRP levels (mg/L)[a]	0.52 (0.22-1.11)	0.80 (0.38-2.07)	<.001	<.001	
MetS, yes/no (%)	73/306 (19.3%)	61/168 (26.6%)	.033	.029	.207

*P*1: unadjusted. *P*2: adjusted for age, sex, smoking, and BMI. *P*3: adjusted for age, sex, smoking, BMI, and sICAM1 or CRP levels.
[a] C-reactive protein levels are presented with median and (interquartile range).

sICAM-I levels, and the highest prevalence of MetS. Subjects with combined *CRP* rs3091244 CC genotype and *ICAM1* rs5491 TT genotype were associated with low hsCRP and high sICAM-I levels, and intermediate risk for MetS. Subjects with *ICAM1* rs5491 AT + AA genotype were associated with low hsCRP and the lowest sICAM-I levels, and thus the lowest prevalence of MetS. Multivariate logistic regression analysis with adjustments for age, sex, smoking, components of MetS, HOMA-IR index, antihypertensive drugs, antidiabetic drugs, lipid-lowering drugs, hsCRP, and sICAM-I levels revealed that the combined *CRP* rs3091244 non-CC genotype and *ICAM1* rs5491 TT genotype carriers were significantly associated with an increased risk of MetS (OR = 2.54; 95% CI, 1.33-4.86; $P = .005$). Similar results were obtained when the International Diabetes Federation definition of MetS was used (OR = 2.05; 95% CI, 1.10-3.82; $P = .023$). Subgroup analysis showed that central obesity interacted with the association between combined *ICAM1* rs5491 and *CRP* rs3091244 polymorphisms and MetS ($P < .001$). Although significant associations between combined genotypes and sICAM1 and CRP levels were noted in both subgroups of subjects (data not shown), associations of combined genotypes with insulin resistance ($P = .039$) and MetS ($P = .001$) were statistically significant only in subjects with central obesity.

4. Discussion

This study investigated the effects of polymorphisms within inflammatory marker genes upon the susceptibility for MetS. The data showed that functional variants on the *CRP* and *ICAM1* genes were associated with MetS, in part because of their effects on the levels of the respective serum markers. The associations were strengthened by combined genotype analysis, in which combined *ICAM1-CRP* genotypes were independently associated with the risk of MetS in multivariate analysis. In subgroup analysis, the combined genotypes were associated with insulin resistance and MetS predominantly in subjects with central obesity. These results revealed that inflammatory gene polymorphisms play an important role in altering the risk for MetS in subjects with central obesity.

Register et al [27] described a case homozygous for $ICAM1^{Kilifi}$ with very low sICAM1 levels. The present investigation further showed that the $ICAM1^{Kilifi}$ is associated with significantly lower levels of sICAM1. We have demonstrated that the *CRP* rs3091244 polymorphism is also independently associated with increased CRP levels in this study population in a previous study [21]. In this investigation, we have combined 2 functional variants from 2 candidate genes to analyze their associations with MetS. So far, case-control studies for MetS have focused on the investigation of single functional candidates to identify low-penetrance susceptibility genes for MetS. It is possible that the combination of several polymorphisms in various genes associated with a biological pathway might significantly influence the risk of MetS, a complex syndrome with multiple components. The pathway-based multigenic approach that combines multiple polymorphisms interacting within a common pathway may amplify the effect of individual polymorphisms and enhance the predictive power [28]. The interaction and additive effects may be a direct result of biological interactions between these specific SNPs or, alternatively, may indirectly reflect other SNPs that are in linkage disequilibrium with these genotyped SNPs. In this study, by combining 2 *ICAM1* and *CRP* gene functional SNPs, our data revealed 3 different subgroups of individuals

Table 5
Association of combined *ICAM1* rs5491 and *CRP* rs3091244 polymorphisms with MetS (ATP-III, Asian criteria)

	CRP–non-CC and *ICAM1*-TT genotype (212)	*CRP*-CC and *ICAM1*-TT genotype (347)	*ICAM1*-AT + AA genotype (47)	*P*1 value	*P*2 value
sICAM1 levels (ng/mL)	250.6 ± 99.7	249.1 ± 116.2	138.5 ± 50.8	<.001	
CRP levels (mg/L)[a]	0.78 (0.36-2.01)	0.52 (0.22-1.11)	0.70 (0.35-1.72)	<.001	
MetS, yes/no (%)	61/151 (28.8%)	69/278 (19.9%)	4/43 (8.5%)	.003	.001

*P*1: unadjusted. *P*2: adjusted for age, sex, smoking, and BMI.
[a] C-reactive protein levels are presented with median and (interquartile range).

with high-high, high-low, and low-low levels of sICAM1 and CRP, revealing a high, intermediate, and low prevalence of MetS. Combined multiple SNPs may therefore provide a more powerful tool in elucidating the molecular genetics of MetS.

Accumulating evidence suggests that the proinflammatory state participates in the progression of MetS. Central obesity and insulin resistance, the central components of MetS, feature a state of chronic low-grade inflammation [1]. Inflammatory parameters may be regarded as additional metabolic criteria that appear to be related to MetS. In an earlier review, Dadona et al suggested that genetic factors may contribute to the inflammatory stress in MetS [29]. To the best of our knowledge, this is the first report revealing combined inflammatory marker gene polymorphisms as genetic risk factors for MetS. Our results further strengthen the roles of chronic inflammation in MetS. Adipose tissue inflamed with macrophages in an undifferentiated state is an underlying problem in MetS and atherosclerosis [30]. This is a possible reason why both the *ICAM1* and *CRP* SNPs are associated with MetS, but neither is associated with any individual component of MetS in our study. Adipose tissue has attracted a great deal of attention as a pathogenic site of obesity-induced insulin resistance, partly because adipose tissue produces bioactive proteins that are readily detected and reflect the inflammatory state of the organ [3]. Because the bulk of accumulated lipid is stored in adipocytes, it is generally assumed that adipocytes initiate the process and macrophages serve to amplify the signal [3]. In this study, combined *ICAM1* and *CRP* genotypes were associated with insulin resistance and MetS in subjects with central obesity but not in subjects without central obesity. These results therefore support the theory that subjects with the appropriate genetic background of elevated inflammatory states may accentuate and propagate adipose tissue inflammation, which results in further insulin resistance and associated MetS.

A limitation of this study was the relatively young age of the sample, and it is unknown whether inflammatory gene polymorphisms affect the risk of MetS in elderly populations. Moreover, we did not stratify subjects with *ICAM1* rs5491 AT + AA genotype according to *CRP* rs3091244 genotypes because of small case numbers in this group. Another limitation of this study is its cross-sectional design, which provided no information about the effect of the inflammatory marker gene polymorphisms on the progression of MetS or clinical outcome. Finally, the examined subjects were ethnically Chinese; and caution should be exercised when extrapolating our results to other ethnic groups.

In conclusion, this investigation demonstrated that inflammatory marker gene polymorphisms play an important role in modulating the risk of insulin resistance and MetS for subjects with central obesity. These results suggest that obese subjects with a genetic background associated with a proinflammatory state may have increased risks for insulin resistance and MetS. These findings also contribute to a better understanding of the mechanism of association between inflammatory markers and the risk of developing atherosclerotic disease.

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References

[1] de Luca C, Olefsky JM. Inflammation and insulin resistance. FEBS Lett 2008;582:97-105.
[2] Després JP, Lemieux I. Abdominal obesity and metabolic syndrome. Nature 2006;444:881-7.
[3] Shoelson SE, Lee J, Goldfine AB. Inflammation and insulin resistance. J Clin Invest 2006;116:1793-801.
[4] Calabro P, Yeh ET. Intra-abdominal adiposity, inflammation, and cardiovascular risk: new insight into global cardiometabolic risk. Curr Hypertens Rep 2008;10:32-8.
[5] Ridker PM, Buring JE, Cook NR, Rifai N. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. Circulation 2003;107:391-7.
[6] Hsu LA, Ko YL, Wu S, et al. Association of soluble intercellular adhesion molecule–1 with insulin resistance and metabolic syndrome in Taiwanese. Metabolism 2009;58:983-8.
[7] Szalai AJ, Wu J, Lange EM. Single-nucleotide polymorphisms in the C-reactive protein (CRP) gene promoter that affect transcription factor binding, alter transcriptional activity, and associate with differences in baseline serum CRP level. J Mol Med 2005;83:440-7.
[8] Staunton DE, Dustin ML, Erickson HP, Springer TA. The arrangement of the immunoglobulin-like domains of ICAM-1 and the binding sites for LFA-1 and rhinovirus. Cell 1990;61:243-4.
[9] Ockenhouse CF, Betageri R, Springer TA, Staunton DE. *Plasmodium falciparum*–infected erythrocytes bind ICAM-1 at a site distinct from LFA-1, Mac-1, and human rhinovirus. Cell 1992;68:63-9.
[10] Berendt AR, McDowall A, Craig AG, et al. The binding site on ICAM-1 for *Plasmodium falciparum*–infected erythrocytes overlaps, but is distinct from, the LFA-1-binding site. Cell 1992;68:71-81.
[11] Duperray A, Languino LR, Plescia J, et al. Molecular identification of a novel fibrinogen binding site on the first domain of ICAM-1 regulating leukocyte-endothelium bridging. J Biol Chem 1997;272:435-41.
[12] Bella J, Kolatkar PR, Marlor CW, Greve JM, Rossmann MG. The structure of the two amino-terminal domains of human ICAM-1 suggests how it functions as a rhinovirus receptor and as an LFA-1 integrin ligand. Proc Natl Acad Sci U S A 1998;95:4140-5.
[13] Casasnovas JM, Stehle T, Liu JH, Wang JH, Springer TA. A dimeric crystal structure for the N-terminal two domains of intercellular adhesion molecule–1. Proc Natl Acad Sci U S A 1998;95:4134-9.
[14] Tsakadze NL, Zhao Z, D'Souza SE. Interactions of intercellular adhesion molecule–1 with fibrinogen. Trends Cardiovasc Med 2002; 12:101-8.
[15] Fernandez-Reyes D, Craig AG, Kyes SA, et al. A high frequency African coding polymorphism in the N-terminal domain of ICAM-1 predisposing to cerebral malaria in Kenya. Hum Mol Genet 1997;6: 1357-60.
[16] Craig A, Fernandez-Reyes D, Mesri M, et al. A functional analysis of a natural variant of intercellular adhesion molecule–1 (ICAM-1Kilifi). Hum Mol Genet 2000;9:525-30.
[17] Brull DJ, Serrano N, Zito F, et al. Human CRP gene polymorphism influences CRP levels: implications for the prediction

and pathogenesis of coronary heart disease. Arterioscler Thromb Vasc Biol 2003;23:2063-9.

[18] Carlson CS, Aldred SF, Lee PK, et al. Polymorphisms within the C-reactive protein (CRP) promoter region are associated with plasma CRP levels. Am J Hum Genet 2005;77:64-77.

[19] Crawford DC, Sanders CL, Qin X, et al. Genetic variation is associated with C-reactive protein levels in the Third National Health and Nutrition Examination Survey. Circulation 2006;114:2458-65.

[20] Kathiresan S, Larson MG, Vasan RS, et al. Contribution of clinical correlates and 13 C-reactive protein gene polymorphisms to interindividual variability in serum C-reactive protein level. Circulation 2006; 113:1415-23.

[21] Teng MS, Hsu LA, Wu S, Chang HH, Chou HH, Ko YL. Association between C-reactive protein gene haplotypes and C-reactive protein levels in Taiwanese: interaction with obesity. Atherosclerosis 2009; 204:e64-9.

[22] Kun JF, Klabunde J, Lell B, et al. Association of the ICAM-1Kilifi mutation with protection against severe malaria in Lambaréné, Gabon. Am J Trop Med Hyg 1999;61:776-9.

[23] Tan CE, Ma S, Wai D, Chew SK, Tai ES. Can we apply the National Cholesterol Education Program Adult Treatment Panel definition of the metabolic syndrome to Asians? Diabetes Care 2004; 27:1182-6.

[24] Chang PY, Wu TL, Tsao KC, Li CC, Sun CF, Wu JT. Microplate ELISAs for soluble VCAM-1 and ICAM-1. Ann Clin Lab Sci 2005;35: 312-7.

[25] Wu TL, Chen Tsai I, Chang PY, et al. Establishment of an in-house ELISA and the reference range for serum amyloid A (SAA): complementarity between SAA and C-reactive protein as markers of inflammation. Clin Chim Acta 2007;376:72-6.

[26] Grundy SM, Cleeman JI, Daniels SR, et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. Circulation 2005;11:2735-52.

[27] Register TC, Burdon KP, Lenchik L, et al. Variability of serum soluble intercellular adhesion molecule-1 measurements attributable to a common polymorphism. Clin Chem 2004;50:2185-7.

[28] Anderson JL, Carlquist JF, Horne BD, Hopkins PN. Progress in unraveling the genetics of coronary artery disease and myocardial infarction. Curr Atheroscler Rep 2007;9:179-86.

[29] Dandona P, Aljada A, Chaudhuri A, Mohanty P, Garg R. Metabolic syndrome: a comprehensive perspective based on interactions between obesity, diabetes, and inflammation. Circulation 2005;111:1448-54.

[30] Gustafson B, Hammarstedt A, Andersson CX, Smith U. Inflamed adipose tissue: a culprit underlying the metabolic syndrome and atherosclerosis. Arterioscler Thromb Vasc Biol 2007;27:2276-83.