TRIB3 Functional Q84R Polymorphism Is a Risk Factor for Metabolic Syndrome and Carotid Atherosclerosis

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OBJECTIVE — To determine the association of *TRIB3* Q84R polymorphism with metabolic syndrome (MetS) and carotid atherosclerosis.

RESEARCH DESIGN AND METHODS — A case-control study enrolled 513 Chinese subjects in three groups: control, MetS, and obese. The functional *TRIB3* Q84R polymorphism was genotyped among subjects undergoing carotid ultrasonography. The clinical and biochemical characteristics were determined.

RESULTS — For individuals with the *TRIB3* R84 allele, the odds ratio for developing MetS was 2.349 ($P = 0.018$), abdominal obesity 2.351 ($P = 0.012$), hypertriglyceridemia 2.314 ($P = 0.00003$), and insulin resistance 1.697 ($P = 0.023$). Likewise, the odds ratio for individuals with the *TRIB3* R84 allele to develop thickened intima-media thickness was 2.208 ($P = 0.040$).

CONCLUSIONS — Individuals with the functional *TRIB3* Q84R polymorphism are at risk for MetS. The *TRIB3* R84 allele especially predisposes to carotid atherosclerosis in part through the effects of abdominal obesity, hypertriglyceridemia, and insulin resistance.

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Metabolic syndrome (MetS) is a powerful and prevalent predictor of cardiovascular events (1,2). Insulin resistance is recognized as the triggering factor of MetS. The *TRIB3* gene, located on chromosome 20p13, has been implicated in insulin resistance (3). A *TRIB3* Q84R polymorphism has also recently been associated with early-onset type 2 diabetes (4). This polymorphism may identify individuals at risk for insulin resistance and related cardiovascular risk (5). Whether the *TRIB3* Q84R polymorphism increases the risk for MetS and carotid atherosclerosis remains to be established.

RESEARCH DESIGN AND METHODS

— A total of 513 unrelated Chinese subjects aged 24–85 years were recruited from the Qilu Hospital of Shandong University: 217 subjects with MetS, defined by the International Diabetes Federation (6); 200 subjects (control subjects) without any abnormality; and 96 obese-alone subjects. Written informed consent was obtained from all subjects, and procedures were approved by the institutional ethics committees.

The clinical and biochemical characteristics of the subjects were determined. Insulin resistance was assessed by the homeostasis model assessment equation (7). Genotyping of the *TRIB3* R84 variant was as previously described (5).

B-mode ultrasonography of the carotid arteries was performed by one trained clinical technician. Both the right and left common carotid arteries were examined. Intima-media thickness (IMT) of the common and internal carotid arteries and bifurcations were measured according to the Asymptomatic Carotid Artery Plaque Study (ACAPS) protocol (8). (For details, see the online-only appendix available at http://care.diabetesjournals.org/cgi/content/full/dc09-0061/DC1.)

Statistical analysis

The Kolmogorov-Smirnov test was used to test for normal distribution. Normally distributed data are presented as means ± SD. Continuous variables were compared among groups by one-way ANOVA with post hoc least-significant differences *t* test or Kruskal-Wallis *H* test. The χ^2 test was used to analyze the associations between categorical variables. Multivariate regression analysis of risk factors was performed, with odds ratios (ORs) (95% CIs) shown. Multiple linear regression analysis was used to evaluate the contribution of risk factors. The correlation between two variables was assessed by Pearson or Spearman correlation analysis. A P value <0.05 was considered significant. Analyses involved SPSS v. 13.0 (SPSS, Chicago, IL).

RESULTS

— Genotyping was successful in 513 case subjects. The *TRIB3* Q84R polymorphism genotypes were in Hardy-Weinberg equilibrium. The clinical and biochemical characteristics of the subjects by *TRIB3* genotype in the MetS group are in Table 1. Subjects with the RR84 genotype had higher waist-to-hip ratio and total cholesterol, triglyceride, and LDL cholesterol levels but lower HDL cholesterol levels than individuals with the

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Table 1—*Clinical and biochemical characteristics of subjects by* TRIB3 *Q84R genotype in MetS group*

	QQ84 (n = 125)	QR84 (n = 79)	RR84 (n = 13)	P vs. QQ84 group	P vs. QR84 group	P* vs. QQ84 group	P* vs. QR84 group
Sex (male/female)	51/74	39/40	9/4	0.076	0.237		
Age (years)	55.15 ± 9.14	54.71 ± 9.84	56.85 ± 6.40	0.532	0.442		
BMI (kg/m^2)	28.95 ± 4.31	28.80 ± 3.90	29.61 ± 2.80	0.581	0.508		
Systolic blood pressure (mmHg)	152.19 ± 22.11	148.10 ± 20.72	156.85 ± 28.47	0.471	0.187		
Diastolic blood pressure (mmHg)	94.14 ± 14.00	93.35 ± 13.74	91.62 ± 16.05	0.539	0.681		
Waist circumference (cm)	98.01 ± 9.73	97.37 ± 10.42	101.31 ± 8.81	0.259	0.188		
Waist-to-hip ratio	0.93 ± 0.06	0.93 ± 0.06	0.96 ± 0.04	0.041	0.041		
Total cholesterol (mmol/l)	5.29 ± 1.29	5.48 ± 1.10	6.03 ± 0.87	0.038	0.130	0.056	0.198
Triglycerides (mmol/l)	2.04 ± 0.92	2.57 ± 1.64	2.77 ± 1.24	0.047	0.587	0.008	0.847
HDL cholesterol (mmol/l)	1.25 ± 0.30	1.24 ± 0.42	1.04 ± 0.19	0.034	0.047	0.077	0.063
LDL cholesterol (mmol/l)	3.49 ± 0.94	3.58 ± 0.96	4.06 ± 1.07	0.044	0.098	0.033	0.088
Fasting blood glucose (mmol/l)	6.69 ± 2.41	6.64 ± 2.71	6.13 ± 2.47	0.446	0.501		
Insulin (μU/ml)	20.47 ± 10.76	20.04 ± 9.67	19.23 ± 14.68	0.694	0.802		
HOMA-IR	6.30 ± 4.71	5.84 ± 3.22	5.24 ± 4.50	0.389	0.639		
Normal IMT	80	41	2	0.001	0.017		
Mean IMT (mm)	0.75 ± 0.15	0.76 ± 0.18	0.95 ± 0.18	0.00007	0.0002		
Maximum IMT (mm)	1.07 ± 0.69	1.23 ± 0.88	2.44 ± 1.17	0.00000005	0.000002		

Data are means ± SD unless otherwise indicated. *P, adjusted for waist-to-hip ratio, sex, age, and smoking. HOMA-IR, homeostasis model assessment for insulin resistance.

QQ84 genotype ($P < 0.05$ for all). Subjects with different Q84R genotypes did not differ in age, sex, BMI, waist circumference, blood pressure, fasting blood glucose, insulin, and homeostasis model assessment of insulin resistance (Table 1) or medication for diabetes or hypertension. The genetic and biochemical results from MetS and control groups suggest an association of the *TRIB3* Q84R variant with metabolism in humans (see supplemental Table A1 in the online appendix).

TRIB3 Q84R genotype was associated with MetS ($P = 0.021$), and stepwise regression analysis revealed the risk factors for MetS as being the *TRIB3* R84 allele (OR 2.349 [95% CI 1.156–4.775]; $P = 0.018$) and smoking (3.130 [1.172–8.358]; $P = 0.023$).

After dichotomization, the *TRIB3* Q84R genotype was associated with abdominal obesity ($P = 0.048$). Furthermore, stepwise multivariate regression analysis revealed the risk factors for abdominal obesity as being the *TRIB3* R84 allele (OR 2.351 [95% CI 1.210–4.568]; $P = 0.012$), sex (1.113 [1.015–1.220]; $P = 0.023$), and triglyceride level (16.869 [1.389–204.808]; $P = 0.027$).

TRIB3 Q84R genotype was associated with hypertriglyceridemia ($P = 0.005$). Furthermore, stepwise multivariate regression analysis revealed the risk factors for hypertriglyceridemia as being the *TRIB3* R84 allele (2.314 [1.494–3.585]; $P = 0.00003$), waist-to-hip ratio (12,007.348 [18.463–7,808,726]; $P = 0.004$), systolic blood pressure (1.029 [1.007- 1.051]; $P = 0.008$), fasting blood glucose (1.769 [1.276–2.453]; $P = 0.001$), and insulin level (1.205 [1.080–1.344]; $P = 0.001$).

Unexpectedly, fasting blood glucose and fasting insulin level had no association with the *TRIB3* Q84R polymorphism. However, the *TRIB3* Q84R genotype was associated with insulin resistance ($P = 0.040$). Stepwise univariate regression analysis demonstrated the *TRIB3* R84 allele as a risk factor for insulin resistance (1.697 [1.076–2.677]; $P = 0.023$). Thus, carriers of the *TRIB3* R84 allele are more susceptible to MetS, especially for abdominal obesity, hypertriglyceridemia, and insulin resistance.

Accordingly, whether the *TRIB3* R84 allele was associated with atherosclerosis was investigated. Subjects with the RR84 genotype had significantly higher mean and maximal IMT than individuals with the QQ84 and QR84 genotypes ($P < 0.001$ for both). We found risk factors for thickened IMT as being the *TRIB3* R84 allele (2.208 [1.036–4.709]; $P = 0.040$), age (1.137 [1.082–1.195]; $P = 8.3 \times 10^{-10}$), systolic blood pressure (1.025 [1.009–1.041]; $P = 0.002$), and waist-to-hip ratio (878.411 [2.311–333,869.6]; $P = 0.025$). Moreover, multivariate linear regression showed that the *TRIB3* Q84R polymorphism contributed, although slightly, to the variation in IMT ($\beta = 0.099$, $P = 0.026$, supplemental Table A2 in the online appendix).

CONCLUSIONS — This is the first large study that comprehensively identifies the *TRIB3* Q84R polymorphism as a candidate single nucleotide polymorphism for MetS and carotid atherosclerosis. We demonstrated that *TRIB3* R84 allele carriers are at increased risk for MetS and carotid atherosclerosis because they are more susceptible to abdominal obesity, hypertriglyceridemia, and insulin resistance.

Therefore, *TRIB3* is speculated to contribute to MetS via alteration of glucose and fat, with insulin playing a pivotal role. That *TRIB3* R84 was also located on chromosome 20p1 further confirms the association because it is generally assumed that susceptibility loci of obesity and type 2 diabetes were on chromosome 20p (9,10). Based on that, it implies that *TRIB3* contributes to MetS via alteration of glucose and fat. However, The *TRIB3* Q84R polymorphism was not included in the gene chips used by recent type 2 diabetes genome-wide association studies to clarify its contribution (11).

Extensive evidence (1,2,12) shows that the presence of MetS increases the risk of atherosclerosis. Here, we showed that the *TRIB3* Q84R genotype facilitates

the morbidity of carotid atherosclerosis. Because we have found that *TRIB3* R84 allele carriers are at increased risk for abdominal obesity, hypertriglyceridemia, and insulin resistance, all of which have been validated as independent risk factors for carotid atherosclerosis (13–15), these features are the variant's link to carotid atherosclerosis.

In summary, the current findings provide direct evidence that individuals with the RR84 are more susceptible to abdominal obesity, hypertriglyceridemia, and insulin resistance. Therefore, they are at risk for MetS and predisposed to carotid atherosclerosis.

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