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 groups. 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 those 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. Especially, the TRIB3 R84 allele predisposes to carotid atherosclerosis in part through the effects of abdominal obesity, hypertriglyceridemia and insulin resistance.
Metabolic syndrome (MetS) is a powerful and prevalent predictor of cardiovascular events[1,2]. Insulin resistance (IR) 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 (controls) 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. IR was assessed by the homeostasis model assessment (HOMA) 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. IMT of the common and internal carotid arteries (ICAs) and bifurcations were measured according to the ACAPS protocol[8]. (For details, see the online-only appendix available at http://care.diabetesjournals.org.)

Statistical analyses: 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 LSD-t test or Kruskal-Wallis H test. The chi-square test was used to analyze the associations between categorical variables. Multivariate regression analysis of risk factors was performed, with odds ratios (ORs; 95% confidence intervals [CIs]) shown. Multiple linear regression analysis was used to evaluate the contribution of risk factors. The correlation between 2 variables was assessed by Pearson or Spearman correlation analysis. A P value <0.05 was considered significant. Analyses involved SPSS v. 13.0 (SPSS Inc., Chicago, IL).

RESULTS

Genotyping was successful in 513 cases. The TRIB3 Q84R polymorphism genotypes were in Hardy-Weinberg equilibrium.

The clinical and biochemical characteristics of the subjects by TRIB3 genotype in MetS group are in Table 1. Subjects with the RR84 genotype had higher waist-to-hip ratio and total cholesterol, triglyceride, and LDL-C levels but lower HDL-C level than those with the QQ84 genotype (P<0.05 for all). Subjects with different Q84R genotypes did not differ in age, sex, BMI, waist circumference, blood pressure, FBG, insulin, and HOMA-IR (Table 1) or medication for diabetes or hypertension. The genetic and biochemical results from MetS and

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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 (OR, 3.130; 95% CI, 1.172-8.358; $P=0.023$).

After dichotomization, 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 (OR, 1.113; 95% CI, 1.015-1.220; $P=0.023$) and triglyceride level (OR, 16.869; 95% CI, 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 (OR, 2.314; 95% CI, 1.494-3.585; $P=0.00003$), waist-to-hip ratio (OR, 12007.348; 95% CI, 18.463-7808726; $P=0.004$), SBP (OR, 1.029; 95% CI, 1.007-1.051; $P=0.008$), FBG (OR, 1.769; 95% CI, 1.276-2.453; $P=0.001$) and insulin level (OR, 1.205; 95% CI, 1.080-1.344; $P=0.001$).

Unexpectedly, FBG and fasting insulin level were found no association with TRIB3 Q84R polymorphism.

However, TRIB3 Q84R genotype was associated with IR ($P=0.040$). Stepwise univariate regression analysis demonstrated the TRIB3 R84 allele as a risk factor for IR (OR, 1.697; 95% CI, 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 IR.

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 those with the QQ84 and QR84 genotypes ($P<0.001$, for both). We found risk factors for thickened IMT as being the TRIB3 R84 allele (OR, 2.208; 95% CI, 1.036-4.709; $P=0.040$), age (OR, 1.137; 95% CI, 1.082-1.195; $P=8.3\times10^{-10}$), SBP (OR, 1.025; 95% CI, 1.009-1.041; $P=0.002$) and waist-to-hip ratio (OR, 878.411; 95% CI, 2.311-333869.6; $P=0.025$). Moreover, multivariate linear regression showed 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).

DISCUSSION
This is the first large study that comprehensively identifies the TRIB3 Q84R polymorphism as a candidate SNP 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 mellitus were on chromosome 20p[9,10]. Based on that, it implies that TRIB3
contributes to MetS via alteration of glucose and fat. However, The \textit{TRIB3} Q84R polymorphism was not included in the gene chips used by recent type 2 diabetes genome wide association studies to clarify its contribution\cite{11}.

Extensive evidence\cite{1, 2, 12} shows that the presence of MetS increases the risk of atherosclerosis. Here, we showed that the \textit{TRIB3} Q84R genotype facilitates the morbidity of carotid atherosclerosis. Since we have found that \textit{TRIB3} R84 allele carriers are at increased risk for the abdominal obesity, hypertriglyceridemia and insulin resistance, all of which have been validated as independent risk factors for carotid atherosclerosis\cite{13,14,15}, 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.

\textbf{ACKNOWLEDGEMENTS}

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\textbf{Disclosures:} No conflict of interests to disclose.
REFERENCE


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<table>
<thead>
<tr>
<th>Characteristics</th>
<th>QQ84 (n=125)</th>
<th>QR84 (n=79)</th>
<th>RR84 (n=13)</th>
<th>P (vs QQ84 group)</th>
<th>P (vs QR84 group)</th>
<th>P (vs QQ84 group)</th>
<th>P (vs QR84 group)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (male/female)</td>
<td>51/74</td>
<td>39/40</td>
<td>9/4</td>
<td>0.076</td>
<td>0.237</td>
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<tr>
<td>Age (years)</td>
<td>55.15±9.14</td>
<td>54.71±9.84</td>
<td>56.85±6.40</td>
<td>0.532</td>
<td>0.442</td>
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<td>BMI (kg/m²)</td>
<td>28.95±4.31</td>
<td>28.80±3.90</td>
<td>29.61±2.80</td>
<td>0.581</td>
<td>0.508</td>
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<tr>
<td>SBP (mmHg)</td>
<td>152.19±22.11</td>
<td>148.10±20.72</td>
<td>156.85±28.47</td>
<td>0.471</td>
<td>0.187</td>
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<td>DBP (mmHg)</td>
<td>94.14±14.00</td>
<td>93.35±13.74</td>
<td>91.62±16.05</td>
<td>0.539</td>
<td>0.681</td>
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<td>WC (mm)</td>
<td>98.01±9.73</td>
<td>97.37±10.42</td>
<td>101.31±8.81</td>
<td>0.259</td>
<td>0.188</td>
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<td>WHR</td>
<td>0.93±0.06</td>
<td>0.93±0.06</td>
<td>0.96±0.04</td>
<td>0.041</td>
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<td>TC (mmol/L)</td>
<td>5.29±1.29</td>
<td>5.48±1.10</td>
<td>6.03±0.87</td>
<td>0.038</td>
<td>0.130</td>
<td>0.056</td>
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<tr>
<td>TG (mmol/L)</td>
<td>2.04±0.92</td>
<td>2.57±1.64</td>
<td>2.77±1.24</td>
<td>0.047</td>
<td>0.587</td>
<td>0.008</td>
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<td>HDL-C (mmol/L)</td>
<td>1.25±0.30</td>
<td>1.24±0.42</td>
<td>1.04±0.19</td>
<td>0.034</td>
<td>0.047</td>
<td>0.077</td>
<td>0.063</td>
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<tr>
<td>LDL-C (mmol/L)</td>
<td>3.49±0.94</td>
<td>3.58±0.96</td>
<td>4.06±1.07</td>
<td>0.044</td>
<td>0.098</td>
<td>0.033</td>
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<td>FBG (mmol/L)</td>
<td>6.69±2.41</td>
<td>6.64±2.71</td>
<td>6.13±2.47</td>
<td>0.446</td>
<td>0.501</td>
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<tr>
<td>Insulin (U/mL)</td>
<td>20.47±10.76</td>
<td>20.04±9.67</td>
<td>19.23±14.68</td>
<td>0.694</td>
<td>0.802</td>
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<td>HOMA-IR</td>
<td>6.30±4.71</td>
<td>5.84±3.22</td>
<td>5.24±4.50</td>
<td>0.389</td>
<td>0.639</td>
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<td>Normal IMT</td>
<td>80</td>
<td>41</td>
<td>2</td>
<td></td>
<td></td>
<td>0.001</td>
<td>0.017</td>
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<tr>
<td>Mean IMT (mm)</td>
<td>0.75±0.15</td>
<td>0.76±0.18</td>
<td>0.95±0.18</td>
<td>0.00007</td>
<td>0.0002</td>
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<tr>
<td>Max IMT (mm)</td>
<td>1.07±0.69</td>
<td>1.23±0.88</td>
<td>2.44±1.17</td>
<td>0.000000005</td>
<td>0.00002</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Data are means ± SD unless otherwise indicated. *P*, adjusted for WHR, gender, age and smoking.

Abbreviations: BMI, body mass index; WC, Waist circumference; WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure; TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; FBG, fasting blood glucose; HOMA-IR, homeostasis model assessment for insulin resistance; IMT, intima-media thickness.