**TRIB1 and GCKR Polymorphisms, Lipid Levels, and Risk of Ischemic Heart Disease in the General Population**

Anette Varbo, Marianne Benn, Anne Tybjærg-Hansen, Peer Grande, Børge G. Nordestgaard

**Objective**—The goal of this study was to test whether TRIB1-rs2954029 and GCKR-rs1260326 associate with lipid levels and risk of ischemic heart disease (IHD) and myocardial infarction (MI) in the general population.

**Methods and Results**—We genotyped >71,000 individuals. Lipid levels were studied cross-sectionally. Risk of IHD and MI was examined prospectively, and in a case-control study, and a metaanalysis was performed. TRIB1 TA (50%) and AA (27%) versus TT (23%) genotypes were associated with increased levels of triglycerides (total increase, +0.16 mmol/L; trend, \( P<0.001 \)), remnant cholesterol (+0.07 mmol/L; \( P<0.001 \)), apolipoprotein B (+5.7 mg/dL; \( P<0.001 \)), and low-density lipoprotein cholesterol (+0.11 mmol/L; \( P<0.001 \)) and with decreased levels of high-density lipoprotein cholesterol (−0.04 mmol/L; \( P<0.001 \)). In metaanalyses of the 3 studies combined, TRIB1 TA and AA versus TT genotypes were associated with 13% (95% CI, 5% to 20%) and 15% (7% to 23%) increased risk of IHD, and 11% (1% to 21%) and 17% (6% to 30%) increased risk of MI, respectively. Although GCKR CT (46%) and TT (14%) versus CC (40%) genotypes had effects on triglycerides (+0.17 mmol/L; trend, \( P<0.001 \)), remnant cholesterol (+0.07 mmol/L; \( P<0.001 \)), and apolipoprotein B (+4.6 mg/dL; \( P<0.001 \)) similar to those of TRIB1, GCKR did not influence low-density lipoprotein cholesterol levels or risk of IHD or MI. Risks of IHD were similar after stratification for gender, age, body mass index, hypertension, diabetes mellitus, smoking, statin use, alcohol intake, and physical activity.

**Conclusion**—In the general population, both TRIB1-rs2954029 and GCKR-rs1260326 were associated with lipid levels, whereas TRIB1 was also associated with increased risk of IHD and MI. (Arterioscler Thromb Vasc Biol. 2011;31:451-457.)

**Key Words:** cardiovascular disease ■ genetics ■ lipoprotein remnants ■ nonfasting ■ triglycerides

Recent genome-wide association studies and a metaanalysis thereof identified polymorphisms in tribbles homolog 1 (TRIB1) and glucokinase regulatory protein (GCKR) as associated with elevated triglyceride levels; however, the influence of these genetic variants on lipid, lipoprotein, and apolipoprotein levels and on risk of ischemic heart disease (IHD) and myocardial infarction (MI) in the general population is unclear.

Elevated fasting and nonfasting triglycerides are associated with increased risk of cardiovascular disease, possibly because elevated triglycerides are a marker of elevated remnant cholesterol, that is, cholesterol in very-low-density lipoprotein remnants in the fasting state, and cholesterol in very-low-density lipoprotein and chylomicron remnants in the nonfasting state. Remnant cholesterol, like low-density lipoprotein (LDL) cholesterol, may be atherogenic by accumulating in the arterial wall. However, elevated fasting and nonfasting triglycerides are also associated with reduced levels of high-density lipoprotein (HDL) cholesterol, which is suggested to mark reduced removal of cholesterol from the arterial wall. Apolipoprotein B is the main protein in LDL and remnants combined, whereas apolipoprotein A1 is the main protein in HDL.

We tested the hypothesis that TRIB1-rs2954029 and GCKR-rs1260326 are associated with levels of nonfasting triglycerides, remnant cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein B, and apolipoprotein A1 and with risk of IHD and MI in the general population. To test this hypothesis, we genotyped more than 71,000 individuals from a prospective population-based study, the Copenhagen City Heart Study (CCHS; \( n=10,598 \)); a cross-sectional population-based study, the Copenhagen General Population Study (CGPS; \( n=40,180 \)); and a population-based case-control study, the Copenhagen Ischemic Heart Disease Study (CIHDS; \( n=20,737 \)). We analyzed data on risk of IHD and MI in each of the 3 studies separately and in a metaanalysis of the 3 studies combined.

**Methods**

**Subjects**

Studies were approved by institutional review boards and Danish ethical committees (Nos. KF-V.100.2039/91, KF-01-144/01, H-KF-...
Blood samples for DNA extraction were available on 10 598
questionnaire, a physical examination, and from blood samples. 

Strong linkage disequilibrium between genotyped for the ABI Prism 7900HT (Applied Biosystems); the CCHS was also 
126 560 154 on the forward strand) and
GCKR-rs1260326 (chromosome 8, position 8q22.1) and
0.91), the CGPS and 
metaanalyses were performed summarizing associations between 
pared using a likelihood ratio test for interaction. 

Multifactorial adjustment was for gender, age, body mass index, hypertension, diabetes mellitus, smoking, and statin use. For all studies, risk estimates adjusted for age and gender only were similar to multifactorially adjusted hazard and odds ratios. 

Association between genotypes and risk of IHD in different strata based on other cardiovascular risk factors in the population was analyzed by logistic regression analysis in the 3 study populations combined to obtain maximal statistical power. Multifactorial adjustment was for gender, age, body mass index, hypertension, diabetes mellitus, smoking, and statin use, with the parameter stratified on excluded from the adjustment. Risk estimates in strata were compared using a likelihood ratio test for interaction.

To summarize results from the 3 independent study populations, metaanalyses were performed summarizing associations between TRIB1-rs2954029 and GCKR-rs1260326 genotypes on risk of IHD and MI. \( F \) statistics evaluated study heterogeneity. Fixed effects models were used, and the metaanalysis was based on variance weighting. For the CCHS, number of cases is slightly larger in the metaanalysis than the number reported in the prospective study, because in the metaanalysis we included both incident and prevalent cases.

Other Covariates

Body mass index was measured weight (kg) divided by measured height squared (m\(^2\)). Hypertension, diabetes mellitus, and smoking were dichotomized and defined as hypertensives (systolic blood pressure, \( \geq 140 \) mm Hg [\( \geq 135 \) mm Hg for diabetics]; diastolic blood pressure, \( \geq 90 \) mm Hg [\( \geq 85 \) mm Hg for diabetics]; or use of antihypertensive medication prescribed specifically for hypertension), diabetics (self-reported disease, use of antidiabetic medication, a nonfasting plasma glucose >11.0 mmol/L, or hospitalization or death due to diabetes [ICD8: 249, 250; ICD10: E10, E11, E13, E14]), and smokers as current smokers. Use of statins was self-reported. Alcohol consumption was self-reported in units per week (1 U = 12 g). Physical activity was coded as low (<2 hours/week), moderate (2 to 4 hours/week), or high (>4 hours/week), depending on how physically active the participants were in their jobs and in their spare time.

Statistical Analysis

Data were analyzed using Stata/SE, version 10.1. Two-sided probability values <0.05 were considered significant. The Student t-test and Pearson \( \chi^2 \)-test were used in 2-group comparisons. One-way analysis of variance was used to compare lipid, lipoprotein, and apolipoprotein levels as a function of genotypes in the CGPS, including controls from the CIHDS; participants with former or current statin use were excluded in these analyses. Nonnormally distributed variables were log transformed to approach normal distribution.

In the prospective CCHS, with the use of left truncation and delayed entry, Cox proportional hazards regression models with age as time scale estimated hazard ratios; individuals diagnosed with an end point before entry were excluded from Cox regression analyses, and those dying during follow-up were censored at their death date. In the cross-sectional CGPS and in the case-control CIHDS, logistic regression analysis was used to estimate odds ratios. Multifactorial adjustment was for gender, age, body mass index, hypertension, diabetes mellitus, smoking, and statin use. For all studies, risk estimates adjusted for age and gender only were similar to multifactorially adjusted hazard and odds ratios.

Association between genotypes and risk of IHD in different strata based on other cardiovascular risk factors in the population was analyzed by logistic regression analysis in the 3 study populations combined to obtain maximal statistical power. Multifactorial adjustment was for gender, age, body mass index, hypertension, diabetes mellitus, smoking, and statin use, with the parameter stratified on excluded from the adjustment. Risk estimates in strata were compared using a likelihood ratio test for interaction.

To summarize results from the 3 independent study populations, metaanalyses were performed summarizing associations between TRIB1-rs2954029 and GCKR-rs1260326 genotypes on risk of IHD and MI. \( F \) statistics evaluated study heterogeneity. Fixed effects models were used, and the metaanalysis was based on variance weighting. For the CCHS, number of cases is slightly larger in the metaanalysis than the number reported in the prospective study, because in the metaanalysis we included both incident and prevalent cases.

Results

The Table shows characteristics of the participants in the 3 independent studies from the Danish population. Compared with the CGPS, participants in the CCHS and the CIHDS were older and were more often male, smokers, and diabetics. Genotype distributions for all study populations were in Hardy-Weinberg equilibrium (all probability values \(>0.8\)).
Lipids, Lipoproteins, and Apolipoproteins

Data on lipid, lipoprotein, and apolipoprotein levels as a function of genotype is shown for the CGPS, including the controls in the CIHDS (n = 40,180 + 15,552) (Figure 1); participants using lipid lowering therapy were omitted from the analysis (n = 5,423), leaving 50,309 participants. Data from the CCHS showed similar patterns (data not shown).

For TRIB1-rs2954029 from TT through TA to AA genotypes, there was a stepwise increase in levels of triglycerides (total increase of 0.16 mmol/L; trend, P < 0.001), remnant cholesterol (+0.07 mmol/L; P < 0.001), LDL cholesterol (+0.11 mmol/L; P < 0.001), and apolipoprotein B (+5.7 mg/dL; P < 0.001), whereas HDL cholesterol levels decreased stepwise (−0.04 mmol/L; P < 0.001) (Figure 1). Apolipoprotein A1 levels did not differ among TRIB1 genotypes (P = 0.56).

For GCKR-rs1260326 CC through CT to TT genotypes, there was also a stepwise increase in levels of triglycerides (total increase of +0.17 mmol/L; trend, P < 0.001), remnant cholesterol (+0.07 mmol/L; P < 0.001), and apolipoprotein B (+4.6 mg/dL; P < 0.001), but LDL cholesterol did not differ among CC, CT and TT genotypes (P = 0.05) (Figure 1). HDL cholesterol levels decreased stepwise for GCKR-rs1260326 (−0.01 mmol/L; P = 0.02), but not as pronounced as for TRIB1-rs2954029, whereas apolipoprotein A1 showed a stepwise increase (+2.3 mg/dL; P < 0.001).

IHD and MI

For IHD in the prospective CCHS, TRIB1-rs2954029 TA and AA versus TT genotypes associated with hazard ratios of 1.22 (95% CI, 1.07 to 1.38) and 1.30 (1.12 to 1.50; trend, P = 0.001) (Figure 2). Corresponding odds ratios were 1.01 (0.91 to 1.11) and 1.02 (0.91 to 1.14) in the CGPS (trend, P = 0.75), and 1.25 (1.11 to 1.41) and 1.23 (1.07 to 1.41) in the CIHDS (trend, P = 0.006), respectively.

For MI in the prospective CCHS, TRIB1-rs2954029 TA and AA versus TT genotypes were associated with hazard ratios of 1.18 (95% CI, 0.98 to 1.44) and 1.41 (1.14 to 1.74; trend, P = 0.001) (Figure 2). Corresponding odds ratios were 1.00 (0.86 to 1.15) and 1.04 (0.88 to 1.22) in the CGPS (trend, P = 0.63), and 1.20 (1.03 to 1.39) and 1.19 (1.01 to 1.41) in the CIHDS (trend, P = 0.05), respectively.

GCKR-rs1260326 was not associated with increased risk of IHD or MI in any of the 3 study populations (Figure 2). Also, GCKR-rs780094, in almost complete linkage disequilibrium with GCKR-rs1260326, was not associated with increased risk of IHD or MI in the CCHS (data not shown).

Metaanalysis

In metaanalyses of the 3 independent studies combined, TRIB1 TA and AA versus TT genotypes were associated with a 13% (95% CI, 5% to 20%) and 15% (7% to 23%) increase in risk of IHD and an 11% (1% to 21%) and 17% (6% to 30%) increase in risk of MI, respectively (Figure 3). GCKR-rs1260326 was not associated consistently with increased risk of IHD or MI in the metaanalysis; however, TT versus CC genotypes were associated with a 9% (1% to 18%) increase in risk of IHD.
Risk of IHD Stratified by Covariates

Risk estimates for IHD were largely similar after stratification for gender, age, body mass index, hypertension, diabetes mellitus, smoking, statin use, alcohol intake, and physical activity for TRIB1 and GCKR polymorphisms (Figure 4). In accordance with this, none of the tests of interaction were statistically significant after correction for multiple comparisons (Bonferroni corrected probability value, <0.05/18=0.003).

Discussion

The main findings of the present studies were that in the general population, both TRIB1 and GCKR polymorphisms were associated with lipid levels, whereas TRIB1 was also associated with increased risk of IHD and MI. These 2 polymorphisms have never before been studied in such large general population studies that included association with levels of lipids, lipoproteins, and apolipoproteins and with risk of IHD and MI.

Mechanistically, the increased risk of IHD and MI in TRIB1-rs2954029 TA and AA versus TT genotypes likely is explained by the combined elevation of both LDL cholesterol and remnant cholesterol together with the reduction in HDL cholesterol. Although cholesterol in both LDL and remnants may accumulate in the arterial intima,9–11 reduced HDL cholesterol may mark reduced removal of cholesterol from the arterial intima.12 Such lipoprotein effects may also explain why GCKR-rs1260326 at most is associated with increased risk of IHD for TT versus CC genotypes in the metaanalysis of the 3 studies combined, because this polymorphism is mainly associated with elevated remnant cholesterol (and therefore also with elevated nonfasting triglycerides and apolipoprotein B), but not with elevated LDL cholesterol, and at most only minimally with reduced HDL cholesterol. The combined data cannot, however, exclude the possibility that triglyceride-rich remnant lipoproteins contribute to development of atherosclerosis, IHD, or MI.

In accordance with the present findings on lipids, lipoproteins, and apolipoproteins in humans in the general population, in vivo mouse studies have found that overexpression of TRIB1 results in reduction of plasma cholesterol, triglycer-
ides, very-low-density lipoprotein, LDL, and apolipoprotein B\(^\text{15}\) and that inactivation of \textit{TRIB1} causes mixed hyperlipemia by increasing hepatic lipogenesis and very-low-density lipoprotein secretion.\(^\text{16}\) \textit{TRIB1} encodes tribble-1, a protein with a regulatory effect on mitogen-activated protein kinase.\(^\text{17}\) It may be through this pathway that \textit{TRIB1} affects lipid metabolism, leading to dyslipidemia, but the exact mechanism is still not known. It has also been suggested that \textit{TRIB1} controls chemotaxis and proliferation of smooth muscle cells in the arterial intima, and it may, through this, lead to atherosclerosis independent of lipoproteins.\(^\text{18}\)

\textit{GCKR} encodes glucokinase regulatory protein, which binds to and regulates the enzyme glucokinase, a key enzyme in glucose metabolism. Regulation of glucokinase affects lipogenesis, and functional studies suggest that it is through this pathway that glucokinase regulatory protein affects triglyceride levels.\(^\text{19}\) Other studies have found that \textit{GCKR} is associated with increased levels of triglycerides concurrent with lower levels of fasting plasma glucose,\(^\text{20,21}\) as opposed to the metabolic syndrome, with increased levels of both plasma triglycerides and glucose. Therefore, the lower glucose levels may also help explain the inconsistent association of \textit{GCKR}-rs1260326 with risk of IHD and MI.

Previous studies\(^\text{1,2,20–22}\) have found associations between the 2 polymorphisms and levels of triglycerides, LDL cholesterol, and HDL cholesterol similar to those observed in the present study. A recent metaanalysis by Teslovich et al\(^\text{3}\) including more than 100,000 individuals from 46 genome-wide association studies of variants associated with elevated triglyceride levels reported effect sizes on triglyceride levels.

<table>
<thead>
<tr>
<th>TRIB1 rs2954029</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>TA vs. TT</th>
<th>AA vs. TT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ischemic heart disease</td>
<td>Copenhagen City Heart Study</td>
<td>2,105</td>
<td>8,493</td>
<td>1.13(1.05-1.20)</td>
</tr>
<tr>
<td>Copenhagen General Population Study</td>
<td>4,039</td>
<td>36,144</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Copenhagen Ischemic Heart Disease Study</td>
<td>5,185</td>
<td>15,552</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summary estimate</td>
<td>11,326</td>
<td>60,189</td>
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</tr>
</tbody>
</table>

| Myocardial infarction | Copenhagen City Heart Study | 1,044 | 9,554 | | |
| Copenhagen General Population Study | 1,643 | 38,537 | | |
| Copenhagen Ischemic Heart Disease Study | 2,978 | 15,552 | | |
| Summary estimate | 5,665 | 63,643 | 1.11(1.01-1.21) | 1.17(1.06-1.30) |

<table>
<thead>
<tr>
<th>GCKR rs1260326</th>
<th>CT vs. CC</th>
<th>TT vs. CC</th>
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<td>Copenhagen City Heart Study</td>
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| Summary estimate | 5,665 | 63,643 | 0.97(0.90-1.05) | 0.99(0.89-1.11) |
of +0.06 mmol/L for \( TRIB1\)-rs2954029 and +0.10 mmol/L for \( GCKR\)-rs1260326, similar to our results of a total increase of +0.16 mmol/L for the \( TRIB1\) polymorphism and +0.17 mmol/L for the \( GCKR\) polymorphism. In accordance with our results, Teslovich et al found \( TRIB1\) to be associated with an increased risk of coronary artery disease, but the association between \( GCKR\) and risk of coronary artery disease was more ambiguous. Also, in an Asian Malay cross-sectional study (n = 2932) of another polymorphism in \( TRIB1\) (rs17321515), Tai et al found this polymorphism to be associated with an increased risk of cardiovascular disease.23

Taken together, the present study, evidence from the previous human studies, and evidence from animal studies all implicate \( TRIB1\) as a gene with effect on lipid metabolism and as a gene involved in atherosclerosis, IHD, and MI.

Limitations include that for \( TRIB1\)-rs2954029, we found an association with increased risk of IHD and MI in the CCHS and CIHDS but not in the CGPS. This could be because the CGPS is a younger population with a shorter follow-up time and therefore fewer events. Also, the study has fewer male participants, smokers, and diabetics compared with the CCHS and therefore does not necessarily translate to populations of other ethnicities.

In conclusion, we found that in the general population, both \( TRIB1\) and \( GCKR\) polymorphisms were associated with lipid levels, whereas \( TRIB1\) was also associated with increased risk of IHD and MI.

Acknowledgments

We thank technicians Hanne Damm and Rikke Aamo for expert technical assistance. We are indebted to the staff and participants of the Copenhagen City Heart Study, the Copenhagen General Population Study, and the Copenhagen Ischemic Heart Disease Study for their important contributions.

Sources of Funding

This study was supported by the Danish Medical Research Council, the Danish Heart Foundation, and Chief Physician Johan Boserup and Lise Boserup’s Fund.

Disclosures

None.

References


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Arterioscler Thromb Vasc Biol. 2011;31:451-457; originally published online November 11, 2010;
doi: 10.1161/ATVBAHA.110.216333
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

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