Interaction of the ACE D Allele and the GNB3 825T Allele in Myocardial Infarction

Christoph K. Naber, Johannes Hüsing, Ulrich Wolfhard, Raimund Erbel, Winfried Siffert

Abstract—In polygenetic disorders, such as ischemic heart disease, the investigation of gene-gene interactions rather than determination of single gene effects is crucial to better understand the contribution of genetic factors. The 825T allele of the G-protein β₃-subunit gene (GNB3) associated with enhanced G-protein signaling is a candidate to interact with the angiotensin-converting enzyme (ACE) deletion/insertion (D/I) polymorphism to increase the risk for myocardial infarction (MI). The ACE D/I variant affects the renin-angiotensin system hormones that activate G-protein–coupled receptors. Genotyping at the ACE and GNB3 loci was performed on 585 patients with coronary artery disease with (n=270) or without (n=315) previous MI. Logistic regression analysis demonstrated a significant interaction between the ACE D allele and the GNB3 825T allele (P<0.001). The odds ratio for MI, associated with the 825T allele, was not increased in the presence of the ACE II genotype (OR 0.5; P=0.09) but was significantly higher in 825T allele carriers with the ACE DI genotype (OR 1.9; P=0.01) and further increased in individuals with the ACE DD genotype (OR 2.4; P=0.02). The highest odds ratio was found in homozygous 825T allele carriers with the ACE DD genotype (OR 7.5; P=0.006). Our data suggest a significant interaction of the GNB3 825T allele with the ACE D allele in MI. These hypothesis-generating data may justify larger prospective studies. (Hypertension. 2000;36:986-989.)

Key Words: myocardial infarction ■ genetics ■ signal transduction ■ G proteins ■ angiotensin-converting enzyme

Although coronary artery disease (CAD) and myocardial infarction (MI) are unlikely to be caused by single genetic polymorphisms, few studies have investigated the interaction of susceptibility genes with other genetic or conventional risk factors on MI. Insight into such interactions may result in a significant gain of knowledge about the pathogenesis of MI and may lead to novel risk stratification, prevention, and even therapeutic strategies.

The DD genotype of a deletion/insertion (D/I) polymorphism in the angiotensin-converting enzyme (ACE) gene was initially reported to increase the risk for MI, but subsequent studies yielded conflicting results potentially caused by the heterogeneity of the respective genetic background. ACE mediates the conversion of angiotensin I to angiotensin II (AT-II), and, although this has not yet been rigorously proven, it is commonly assumed that the increased serum ACE levels associated with the ACE D allele represent the main mechanism by which the ACE DD genotype increases the risk for MI. AT-II receptors are typical G-protein–coupled receptors; thus it appears plausible to assume that AT-II mediated effects are further enhanced in the presence of an increased responsiveness of G proteins. Enhanced G-protein reactivity is strictly correlated with the 825T allele of a C825T base exchange in the gene GNB3 encoding the G-protein β₃-subunit. The 825T allele is also associated with essential hypertension and with an enhancement of diverse cell functions that may play a role in mechanisms ultimately contributing to an increased risk for MI. Therefore, we investigated the hypothesis that the ACE D allele and the GNB3 825T allele significantly interact to increase the risk for MI. This analysis was conducted within a sample of thoroughly characterized patients with angiographically documented CAD with or without previous MI.

Methods

This study was conducted in accordance with the Helsinki Declaration, revised in 1983.

Study Population

A total of 585 patients with angiographically confirmed CAD were consecutively enrolled in this study. CAD was defined by a luminal narrowing with >50% diameter stenosis in at least 1 coronary artery. The following variables were assessed: current age, gender, body mass index, hypercholesterolemia, hypertension, diabetes, smoking, and previous MI. Previous MI was confirmed in 270 patients according to the American College of Cardiology/American Heart Association guidelines for the management of acute MI, whereas 315 individuals had no previous MI according to standard laboratory, clinical, ECG, and angiographic criteria. Patients were classified as hypertensive when they had a documented history of hypertension, used blood pressure–lowering drugs, or if repeated systolic and diastolic blood pressure measurements were >140/90 mm Hg. Patients were classified as hypercholesterolemic if serum cholesterol values were >5.2 mmol/L or if the individual received cholesterol-
TABLE 1. Demographic Data

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>n</th>
<th>Male</th>
<th>Female</th>
<th>Age, y</th>
<th>BMI, kg/m²</th>
<th>Hypertension, n (%)</th>
<th>Cholesterolemia, n (%)</th>
<th>Type 2 Diabetes, n (%)</th>
<th>Current Smoking, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No MI</td>
<td>315</td>
<td>248</td>
<td>67</td>
<td>62.2 ± 8.4</td>
<td>26.6 ± 3.6</td>
<td>226 (71.8)</td>
<td>232 (73.7)</td>
<td>56 (17.8)</td>
<td>46 (14.6)</td>
</tr>
<tr>
<td>MI</td>
<td>270</td>
<td>228</td>
<td>42</td>
<td>60.7 ± 9.4</td>
<td>26.6 ± 3.3</td>
<td>193 (71.5)</td>
<td>213 (78.9)</td>
<td>58 (21.5)</td>
<td>56 (20.7)</td>
</tr>
<tr>
<td>P</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

Values are n, n (%), or mean ± SD.

lowering therapy. Patients were classified having type 2 diabetes when receiving antidiabetic therapy or if fasting glucose was >6.993 mmol/L. All participants were white, of German offspring from the area of Essen, Germany.

Determination of Genotypes
Genotyping at the GNB3 and the ACE gene locus was conducted as previously described. 5,17

Statistical Analysis
Analyses were carried out with the SAS software package (version 6.1.2). Hardy-Weinberg equilibrium (HWE) was tested by comparing a likelihood ratio test statistic with a χ² distribution with 1 df. 18 For a preliminary analysis, association for 2 groups was tested by Student’s t test or, where appropriate, by Fisher’s exact test for dichotomous variables. ORs with 95% CIs were calculated as crude ORs by a likelihood ratio test statistic with a χ² distribution with 1 df. 18

For a preliminary analysis, association for 2 groups was tested by Student’s t test or, where appropriate, by Fisher’s exact test for dichotomous variables. ORs with 95% CIs estimate the relative risk for MI associated with the respective genotypes. They were calculated as crude ORs by a χ² test and by logistic regression analysis including gender, age, body mass index (BMI), hypertension, type 2-diabetes, and smoking status as established risk factors for MI. Interaction between genotypes at the ACE locus and GNB3 was estimated within the logistic model. The significance level was set at α=0.05.

Results
Patients with CAD (with and without MI) and individuals with different genotypes (data not shown) were comparable with respect to common risk factors (Table 1). There was no significant association between genotypes and any of the investigated risk factors. No significant deviation from HWE was observed except for a borderline-significant deviation for the C825T genotypes in the MI group (P=0.05), which resulted from a slight lack of homozygous 825T allele carriers (−3%; Table 2). The ACE genotype distribution and D allele frequency were similar in both groups (Table 2), and the OR for MI related to the ACE D allele was not significantly different from 1.0 (Table 3). The frequency of the 825T allele was increased in the group with previous MI (P=0.03; Table 2), and logistic regression analysis yielded slightly increased, significant ORs for MI (OR for TT/CC, 1.5; 95% CI, 1.1 to 2.0; P=0.01) in 825T allele carriers (Table 3).

We observed a significant interaction between genotypes at GNB3 and genotypes at the ACE locus with respect to MI (P<0.001). Subsequently, a stratified analysis was performed with respect to ACE genotypes to study the specific effects of the GNB3 C825T genotypes. GNB3 genotype distribution (P=0.007) and 825T allele frequency (P=0.002) was significantly different between patients with and those without MI within the ACE DD genotype. A significant albeit smaller effect was still observed within the ACE ID genotype but not within the ACE II genotype (Table 2).

Calculation of crude ORs for MI yielded the highest ORs for TT and TC genotypes within the ACE DD genotype (OR for TT/CC, 7.5; 95% CI, 1.5 to 37.3; P=0.006; OR for

TABLE 2. ACE and GNB3 Genotype Distributions in Study Groups

<table>
<thead>
<tr>
<th>Phenotype/Genotype</th>
<th>TT</th>
<th>TC</th>
<th>CC</th>
<th>P</th>
<th>%T</th>
<th>%C</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No MI (n=315)</td>
<td>22</td>
<td>131</td>
<td>162</td>
<td>0.06</td>
<td>27.8</td>
<td>72.2</td>
<td>0.03</td>
</tr>
<tr>
<td>MI (n=270)</td>
<td>23</td>
<td>135</td>
<td>112</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>DD</td>
<td>DI</td>
<td>II</td>
<td>%D</td>
<td>%I</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No MI (n=315)</td>
<td>74</td>
<td>174</td>
<td>67</td>
<td>0.10</td>
<td>51.1</td>
<td>48.9</td>
<td>0.61</td>
</tr>
<tr>
<td>MI (n=270)</td>
<td>71</td>
<td>126</td>
<td>73</td>
<td></td>
<td>49.6</td>
<td>50.4</td>
<td></td>
</tr>
</tbody>
</table>

ACE DD

| No MI (n=74)       | 2    | 27   | 45   | 0.007| 20.9| 79.1| 0.002|
| Mi (n=71)          | 9    | 35   | 27   |     | 37.3| 62.7|     |

ACE DI

| No MI (n=174)      | 14   | 68   | 92   | 0.021| 27.6| 72.4| 0.043|
| Mi (n=126)         | 10   | 69   | 47   |     | 35.3| 64.7|     |

ACE II

| No MI (n=67)       | 6    | 36   | 25   | 0.20 | 35.8| 64.2| 0.10|
| Mi (n=73)          | 4    | 31   | 38   |     | 26.7| 73.3|     |

%T, %C, %D, %I indicate frequency of the respective allele (%).
Values are n (%).
TC/CC, 2.2; 95% CI, 1.1 to 4.3; \( P = 0.03 \)) and the lowest for TT and TC genotypes within ACE II genotype (OR for TT/CC, 0.5; 95% CI, 0.1 to 1.7; \( P = 0.3 \); OR for TT/CC, 0.6; 95% CI, 0.3 to 1.1, \( P = 0.1 \)). Moreover, logistic regression analysis showed an increasing OR for MI related to the GNB3 825T allele from the homozygous ACE II genotype (OR for TT/TC, 0.5; 95% CI, 0.32 to 1.01; \( P = 0.09 \)) over the ACE DI genotype (OR for TT/TC, 1.9; 95% CI, 1.2 to 3.0; \( P = 0.01 \)) to the ACE DD genotype (OR for TT/TC, 2.4; 95% CI, 1.2 to 4.8; \( P = 0.02 \)) (Table 3).

### Discussion

**ACE D Allele and Risk for MI**

In this study, as in others, a recent meta-analysis, however, supports an association of the ACE D allele with MI in whites, which appears stronger in the Japanese.22

**GNB3 825T Allele and Risk for MI**

Several characteristics of 825T allele carriers make a contribution to MI of this variant sensible: (1) the 825T allele was shown to be associated with hypertension and obesity,5-8,23-26 which are associated with the 825T allele in East Asians compared with whites.24

On one hand, the observed interaction may result from a synergistic but independent effect of each genetic factor. On the other hand, functional considerations support the hypothesis of a true interaction between both alleles: hormones of the renin-angiotensin system, which is affected by the ACE DI polymorphism, activate G-protein–coupled receptors, whose signaling properties may be altered in the presence of an 825T allele at GNB3. G-protein \( \beta \gamma \)-subunits play a role in modulating agonist-receptor affinity of the AT-II receptors,29 and AT-II in particular increases the expression of PTX-sensitive G proteins,29 whose signaling properties are in turn enhanced in the presence of an 825T allele at GNB3.5 In addition, several pathways downstream of the AT-II receptors affect signaling cascades in which G protein \( \beta \gamma \)-subunits are assumed to be involved, for example, activation of phospholipases C30 and D,31 regulation of Ca\(^{2+} \) channels,32 and the transactivation of growth factors.33 For example, such an interaction may result in a further enhanced platelet aggregation11,34 with an increased susceptibility for acute coronary thrombosis. Unfortunately, little is known about the specific contribution of G\( \beta \)3 and its splice variant, G\( \beta \)3s, to these processes. Moreover, besides increased ACE levels, an effect of the ACE D allele on AT-II concentration has not yet been proven.

Thus, the molecular nature of the interaction of the GNB3 825T allele with the ACE D allele remains to be elucidated.

### Combined Genotype Analysis

The complex polygenic nature of ischemic heart disease implies that interactions of various candidate genes are substantial to increase the risk for MI. It seems plausible that a gene variant associated with increased serum ACE concentrations (ACE D allele) may become pathophysiologically important in individuals whose genetic makeup comprises an allelic variant that causes an increased cell responsiveness. In this respect, the GNB3 825T allele represents an attractive candidate to interact with the ACE D allele. Following this hypothesis, we observed a significant interaction between the 825T allele and the ACE D allele. To study the specific effects of the GNB3 C825T genotypes, subsequent stratification was performed with respect to the ACE genotypes. We found a significantly increased OR for MI associated with the 825T allele in individuals with the ACE DD genotype, which was particularly high in GNB3 TT genotypes. The increased OR associated with the 825T allele was smaller in individuals with ACE ID genotype and completely disappeared in conjunction with the ACE II genotype.

These results underline the hypothesis that 2 functional allelic variants, each contributing not or only to a minor extent to a common trait, can interact significantly in combined analyses. The more stringent association of the ACE D allele with MI in Japanese individuals22 thus may be partly explained by the significantly elevated frequency of the GNB3 825T allele in East Asians compared with whites.24

**TABLE 3. Odds Ratios for MI for ACE and GNB3 Genotypes**

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>OR</th>
<th>95% CI</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE DD vs II</td>
<td>1.1</td>
<td>0.8–1.7</td>
<td>0.29</td>
</tr>
<tr>
<td>GNB3 TT/TC vs CC</td>
<td>1.5</td>
<td>1.1–2.0</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>(TT vs CC)</td>
<td>(TC vs CC)</td>
<td>(TT/TC vs CC)</td>
</tr>
<tr>
<td>ACE DD</td>
<td>7.5</td>
<td>(1.5–37.3)</td>
<td>2.2</td>
</tr>
<tr>
<td>ACE DI</td>
<td>1.4</td>
<td>(0.6–3.4)</td>
<td>2.0</td>
</tr>
<tr>
<td>ACE II</td>
<td>0.5</td>
<td>(0.1–1.7)</td>
<td>0.6</td>
</tr>
</tbody>
</table>
several comparable genetic association studies. Hence, inherent to study design, our results do not unequivocally rule out a survival advantage for 825T allele carriers in acute MI.

In conclusion, our data suggest a significant interaction and combined contribution of the GNB3 825T allele and the ACE D allele to MI. More investigations into gene-gene interactions is a prerequisite before the implementation of genetic testing into clinical routine diagnostics, as the reproducibility and predictive power is still far too low. The decoding of the entire human genome is soon to come, and novel techniques for assessing sequence variation on a genome scale will prompt comprehensive studies of comparative genomic diversity in human populations. Consequent research in the field of gene-gene and gene-environment interactions will be required to develop diagnostic scores that more precisely predict the individual risk for multifactorial disorders such as MI.

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References


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