Clinical research

Association between the low activity genotype of catechol-\(\text{O}\)-methyltransferase and myocardial infarction in a hypertensive population

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Aim Estrogens regulate several biological processes involved in the pathogenesis of myocardial infarction. Catechol-\(\text{O}\)-methyltransferase (COMT) is a key enzyme in the degradation of estrogens. There is a functional polymorphism in the COMT gene (Val158Met), affecting the activity of the enzyme. We investigated if the low activity genotype of COMT is associated with an altered risk of myocardial infarction.

Methods and results In a prospectively followed hypertensive cohort we identified 174 patients who suffered a myocardial infarction during the study and compared them to 348 controls from the same cohort. The COMT polymorphism and serum levels of sex hormones were analysed. Patients homozygous for the low activity COMT genotype had a decreased risk of myocardial infarction compared to those with the high activity genotype, odds ratio 0.65 (95% CI 0.44–0.97, \(p = 0.033\)). The protective effect of the low activity genotype was most evident among older patients (>58 years of age), odds ratio 0.43 (95% CI 0.23–0.79, \(p = 0.006\)). Serum levels of estradiol were increased (\(p = 0.006\)) in males with the low activity genotype.

Conclusions Our findings suggest that the low activity COMT genotype is protective against myocardial infarction. One may speculate that the altered estrogen status could be involved in this effect.

Covered in this issue

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Keywords
Myocardial infarction; Estrogen; COMT; Polymorphism

Introduction

Ischemic heart disease is a complex disorder in which environmental as well as genetic factors are involved.\(^{1}\) During the last years efforts have been made to elucidate the genetic background of myocardial infarction both using genetic-linkage studies\(^{2}\) and candidate gene analyses.\(^{3}-^{6}\) Epidemiological approaches have indicated that polymorphisms of some candidate genes, including angiotensin-converting enzyme, platelet glycoprotein...
Ill, coagulation factor VII, connexin 37, plasminogen-activator inhibitor type 1 and stromelysin, are associated with an altered risk of myocardial infarction. However, it has been difficult to confirm some of these findings in other studies including patients from different populations and cardiovascular risk groups.

The incidence of myocardial infarction among premenopausal women is lower than among men at the same age. After menopause, the incidence increases and it has been assumed that this is due to the decreasing levels of estrogen. It is well established that estrogens have beneficial effects on serum lipid concentrations. Direct effects of estrogens on blood vessels include nitric oxide mediated vasodilatation and inhibition of the response of blood vessels to injury. Observational studies have shown that hormone replacement therapy (HRT) is associated with a reduced risk of myocardial infarction among postmenopausal women. In contrast, a recent large randomized controlled primary prevention trial demonstrated that treatment with estrogen in combination with progesterin slightly increased the incidence of coronary heart disease in postmenopausal women.

Estrogen has traditionally been considered a female sex steroid but accumulating data indicate that estrogen is of importance also in males.

Estrogens are to a large extent metabolized to catechol estrogens (CE). CE retain some hormonal activity and modulate oxidative stress. The COMT gene codes for the Catechol-O-methyltransferase enzyme, which mediates the conversion of CE to more inactive metabolites. Interestingly, a functional G to A (valine to methionine amino acid change) polymorphism in the fourth exon, codon158, of the COMT gene, differentiating the COMT-H (high activity) and COMT-L (low activity) alleles, results in a 60–75% decreased methylation activity of COMT. Thus, COMT-L/L homozygote individuals have a distinctly decreased COMT activity, resulting in a less efficient inactivation of CE. In a recent report it was demonstrated that the serum estradiol levels, three hours after treatment with estradiol valerate, were increased in postmenopausal women with the low activity COMT genotype compared with women homozygous for the high activity genotype. Exposure to high levels of estrogens plays an important role in the etiology of breast cancer and this effect of estrogens might be exerted either directly by estrogens themselves or by their metabolites, including CE. An association between the COMT-L/L genotype and breast cancer has been reported in some but not all studies. The proposed mechanism for such an association is an increased activity of estrogens and/or CE in patients with this genotype.

COMT is also involved in the degradation of catecholamines and some but not all studies have indicated an association between the COMT-L allele and prefrontal cognitive function and risk of schizophrenia.

Since estrogens exert several important effects on the cardiovascular system and individuals with the COMT-L/L genotype have an affected estrogen metabolism, the aim of the present study was to evaluate if the COMT-L/L genotype is associated with an altered risk of myocardial infarction in a very well characterized prospective nested case-control study from a Swedish hypertensive cohort.

Methods

Patients

CAPP was a large prospective morbidity and mortality study in which the ACE-inhibitor captopril was compared to conventional antihypertensive therapy (diuretics and beta blockers) in 10,985 hypertensive patients who were followed for a mean of 6.1 years. Blood samples were continuously collected from patients after informed consent during the conduction of the CAPP study, and stored at −70 °C. CAPP was performed in Sweden (7511 patients) and in Finland (3476 patients), but blood was collected principally in Sweden, and therefore only Swedish patients were included in the present study. The primary endpoint of CAPP was cardiovascular mortality and morbidity, including myocardial infarction. All possible myocardial infarctions occurring during the trial were assessed by an independent endpoint committee from which the treatment allocation was concealed. A diagnosis of acute myocardial infarction required that at least two of the following criteria were met: central chest pain for more than 15 min; transient increase in serum concentrations of enzymes indicating myocardial damage; and electrocardiographic changes typical of myocardial infarction. The incidence of myocardial infarction, did not differ between the two treatment groups in the trial. 256 individuals in the Swedish cohort of CAPP suffered from at least one myocardial infarction during the study. 44 (17.2%) of these had a fatal myocardial infarction. Blood samples for DNA analysis were available from 174 (68.0%) of the Swedish individuals with myocardial infarction (157 with non-fatal myocardial infarction (74%) and 17 with fatal myocardial infarction (39%)). Among the patients with myocardial infarction, there was no significant difference in the proportion of patients belonging to the captopril treatment group between the patients with low activity COMT genotype and the patients with high activity genotype (data not shown). Thus, we identified 174 patients from the Swedish cohort who had suffered a myocardial infarction during the study follow up and from whom whole blood samples were available. Each patient with myocardial infarction was matched with two other control subjects from the cohort, matched for sex, age and smoking status, and who had not suffered a myocardial infarction during the time until the occurrence of myocardial infarction for the nested patient, providing a total of 174 patients and 348 controls. The study complies with the Declaration of Helsinki and was approved by the Ethics Committee of the University of Gothenburg. Informed consent was obtained from the subjects.

Biochemical analyses

Cholesterol and triglyceride levels were determined by fully enzymatic techniques on a Kanelab 20 autoanalyzer (Thermo Clinical Labsystems, Espoo, Finland). High-density lipoprotein (HDL) was determined after precipitation of apolipoprotein (apo) B-containing lipoproteins with magnesium sulfate and dextran sulfate (Thermo Clinical Labsystems). 17β-estradiol and testosterone were measured by Spectria 125I-Coated Tube Radioimmunassay and sex hormone-binding globulin (SHBG) was measured by IRMA 125I-Immunoendometric Assay (Orion Diagnostica, Espoo, Finland). Free estradiol was calculated as the ratio of total 17β-estradiol to SHBG. A free androgen index (FAI) was calculated as the ratio of total testosterone to SHBG.
DNA Isolation and genotyping

DNA was isolated with the use of a commercial kit (Wizard® DNA Purification Kit, Promega, Madison, WI, USA). Amplification by PCR was performed on a Multiblock System (Hybaid, Middlesex, UK) according to the manufacturers protocol (Hybaid® Limited march 2000 1.0 – Software Version 3.1). The forward and reverse primers for the COMT polymorphism were, respectively, 5'-AGT GGA TCT GAT TGT CGC TTG and 5'-biotin-AGG CACGC ACO CTC TTG CTT, and the annealing temperature was 54°C. The COMT G-A polymorphism was genotyped using the dynamic allele specific hybridization (DASH) method. The following probes were used in the DASH analyses: 5'-TCG CTG GGT GAAGGAC-3, which is specific for the high activity COMT allele and 5'-TCG CTG GCA GT GAAGGAC-3, which is specific for the low activity COMT allele.

Statistical analysis

Continuous variables were compared between patients with myocardial infarction and controls using Wilcoxon signed ranks test and categorical variables were compared with adjusted Mantel–Haenzel tests. Adjusted Mantel–Haenzel test was also used to compare the percentage of individuals with the different COMT alleles between the patients with myocardial infarction and the controls. Crude odds ratios with 95% confidence intervals, as estimates of the relative risk for myocardial infarction for patients with the low activity COMT genotype, were calculated using conditional logistic regression. To adjust for the effects of other cardiovascular risk factors, adjusted odds ratios with 95% confidence intervals were calculated using conditional logistic regression. Diabetes, cholesterol and triglycerides were used as covariates in these analyses of adjusted odds ratio. No model building procedures were used to obtain this model. The matching for age, sex and smoking status was taken into account in the conditional logistic regression models and in the Mantel–Haenzel test. In the logistic regression models, Wald statistics was used for calculating p values.

Serum levels of sex hormones were compared between individuals with the different COMT genotypes using the Mann–Whitney U test. All tests were two-tailed and conducted at a 5% significance level. All values are means ± SD.

Results

The characteristics of the study population are summarized in Table 1. Since patients and controls were matched for age, sex and smoking status these variables did not differ between the two groups. The patients who during study follow up suffered a myocardial infarction were more likely to have diabetes mellitus and had a higher body mass index but lower HDL than controls (Table 1). Serum levels of estradiol did not differ between patients and controls (data not shown). Two women were on HRT at the occurrence of their myocardial infarction and five controls were on HRT at the occurrence of the myocardial infarction of their nested patient. Data on menopausal status were not available.

Genotyping for the COMT polymorphism was successful in 174/174 of the patients and in 348/348 of the controls. The genotype distribution of the entire cohort was in Hardy–Weinberg equilibrium. The allele frequency in the entire cohort was $H = 45.4\%$ for the patients with myocardial infarction, and $H = 41.5\%$ for the matched controls. These allele frequencies are very similar to what has been reported in another Swedish population. Initial analysis, including all three genotypes (COMT H/H, H/L and L/L), demonstrated that the COMT polymorphism was associated with the risk of myocardial infarction ($p < 0.05$). Individuals homozygous for the COMT-L allele (L/L) are considered to have the low activity COMT genotype compared with individuals with the H/H and H/L genotypes, which are regarded as having the high activity genotype. The distribution of the COMT high activity and low activity genotypes for patients suffering a myocardial infarction and controls is shown in Table 2. The prevalence of the low activity genotype was lower among the patients who suffered a myocardial infarction than among the controls (25.9% versus 35.3%, $p = 0.032$, Table 2). Serum levels and metabolism of estrogens are altered by age, supported by the fact that in our cohort free calculated serum levels of estradiol were lower for males above the median age (58 years of age) than for males below the median age (data not shown). Therefore, the association between the low activity COMT genotype and myocardial infarction was also evaluated separately for the subjects below and above the median age. Among the subjects older than 58 years of age the difference in the prevalence for the low activity genotype between the two groups was more pronounced (myocardial infarction 22.0%, controls 40.2%, $p = 0.005$) than among all subjects while no significant difference between the two groups was seen among the younger patients (Table 2).

Conditional logistic regression analyses, taking the matching factors (sex, age smoking) into account, demonstrated that the crude odds ratio for myocardial infarction of all subjects with respect to the low activity COMT genotype was 0.65 (95% CI 0.44–0.97, $p = 0.033$) and for subjects older than 58 years of age the odds ratio was 0.43 (95% CI 0.23–0.79, $p = 0.006$; Table 3). Conditional logistic regression was also used to adjust for known cardiovascular risk factors including diabetes mellitus, cholesterol and triglycerides. The adjusted odds ratio for myocardial infarction with respect to the low activity genotype of all subjects provided by this model was 0.65 (95% CI 0.44–0.98, $p = 0.040$) and for subjects older than 58 years of age, the odds ratio by this model was 0.42 (95% CI 0.22–0.80, $p = 0.009$, Table 3). Inclusion of HRT as an additional covariate did not change the results (data not shown).

One possible mechanism behind the association between the low activity COMT genotype and myocardial infarction is that this genotype results in higher levels of estrogens, which then in turn might exert a cardioprotective effect. We, therefore, analyzed sex hormone levels (Table 4). Interestingly, both total serum levels of estradiol and estradiol adjusted to SHBG were clearly increased in males with the low activity genotype compared with males with the high activity genotype (Table 4). Due to this finding, the association between COMT genotype and myocardial infarction in male subjects only was determined. The crude odds ratio, as determined by conditional logistic regression, for myocardial infarction in all male subjects, with respect to the low activity COMT
genotype, was 0.63 (95% CI 0.40–1.01) and for males >58 years of age it was 0.42 (95% CI 0.20–0.88). The association between COMT genotype and serum levels of estradiol in the matched male control patients only was of borderline significance (Table 4). All described associations between COMT genotype and myocardial infarction in the whole cohort as well as in male subpopulations disappeared when serum levels of estradiol were included in the conditional logistic regression model (data not shown), supporting the notion that altered serum estradiol levels might be involved in the mechanism behind the association between COMT and myocardial infarction. No significant effect on serum levels of estradiol was seen in the smaller and more heterogeneous female subgroup including both pre- and postmenopausal women, some of which were taking HRT (data not shown).

**Discussion**

Since myocardial infarction is a leading cause of death in the Western world, cardiovascular prevention remains an

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Characteristics of the patients with myocardial infarction and the controls</th>
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<tbody>
<tr>
<td>Characteristics</td>
<td>Controls (n = 348)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.0 ± 6.6</td>
</tr>
<tr>
<td>Male sex (%)</td>
<td>74.1</td>
</tr>
<tr>
<td>Smokers (%)</td>
<td>37.4</td>
</tr>
<tr>
<td>Diabetes (%)</td>
<td>7.5</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.2 ± 3.9</td>
</tr>
<tr>
<td>Cholesterol (mM)</td>
<td>6.18 ± 1.09</td>
</tr>
<tr>
<td>HDL (mM)</td>
<td>1.29 ± 0.37</td>
</tr>
<tr>
<td>LDL (mM)</td>
<td>3.93 ± 0.97</td>
</tr>
<tr>
<td>Triglycerides (mM)</td>
<td>2.23 ± 1.37</td>
</tr>
</tbody>
</table>

Values are means ± SD, M denotes matched for this variable.

<table>
<thead>
<tr>
<th>Table 2</th>
<th>High and low activity COMT polymorphism in relation to myocardial infarction</th>
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</thead>
<tbody>
<tr>
<td>Age Genotype</td>
<td>Controls n (%)</td>
</tr>
<tr>
<td>All ages High activity</td>
<td>225 (64.7%)</td>
</tr>
<tr>
<td>Low activity</td>
<td>123 (35.3%)</td>
</tr>
<tr>
<td>Age &gt;58 High activity</td>
<td>98 (59.8%)</td>
</tr>
<tr>
<td>Low activity</td>
<td>66 (40.2%)</td>
</tr>
<tr>
<td>Age ≤ 58 High activity</td>
<td>127 (69.0%)</td>
</tr>
<tr>
<td>Low activity</td>
<td>57 (31.0%)</td>
</tr>
</tbody>
</table>

MI denotes myocardial infarction; n denotes number of patients; low activity denotes COMT L/L; high activity denotes COMT H/L + H/H.

<table>
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<tr>
<th>Table 3</th>
<th>Odds ratio for myocardial infarction with respect to low activity COMT genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age Crude OR (95% CI)</td>
<td>p Value</td>
</tr>
<tr>
<td>All ages</td>
<td>0.65 (0.44–0.97)</td>
</tr>
<tr>
<td>Age &gt;58</td>
<td>0.43 (0.23–0.79)</td>
</tr>
<tr>
<td>Age ≤ 58</td>
<td>0.93 (0.54–1.59)</td>
</tr>
</tbody>
</table>

OR denotes odds ratio; CI denotes confidence interval; Crude denotes matched for sex, age and smoking status; Adjusted denotes matched for sex, age and smoking status and adjusted for cholesterol, triglycerides and diabetes.

<table>
<thead>
<tr>
<th>Table 4</th>
<th>Sex hormone levels according to low and high activity COMT genotypes in males</th>
</tr>
</thead>
<tbody>
<tr>
<td>All males</td>
<td>Males with MI</td>
</tr>
<tr>
<td>Estradiol (pmol/l)</td>
<td></td>
</tr>
<tr>
<td>High activity (n = 260)</td>
<td>Low activity (n = 121)</td>
</tr>
<tr>
<td>72.5 ± 33.0</td>
<td>81.7 ± 34.8</td>
</tr>
<tr>
<td>Testosterone (nmol/l)</td>
<td></td>
</tr>
<tr>
<td>16.1 ± 6.4</td>
<td>15.2 ± 5.2</td>
</tr>
<tr>
<td>SHBG (nmol/l)</td>
<td></td>
</tr>
<tr>
<td>36.3 ± 17.0</td>
<td>36.2 ± 17.9</td>
</tr>
<tr>
<td>Estradiol/SHBG</td>
<td></td>
</tr>
<tr>
<td>2.51 ± 1.93</td>
<td>2.86 ± 1.93</td>
</tr>
<tr>
<td>FAI (%)</td>
<td></td>
</tr>
<tr>
<td>50.0 ± 23.6</td>
<td>49.6 ± 22.9</td>
</tr>
</tbody>
</table>

Values are means ± SD; low activity denotes COMT L/L; high activity denotes COMT H/L + H/H; n denotes number of patients; FAI denotes testosterone/SHBG in %.
important public health issue. One approach to prevent this condition is to identify disease-susceptibility genes. In our study we have shown that the low activity COMT genotype reduces the risk of myocardial infarction in a prospectively followed cohort of Swedish hypertensive patients. Furthermore, men homozygous for the low activity allele of COMT had increased serum levels of estradiol, which one may speculate to be involved in the cardioprotective effect of this genotype.

In a recent large scale study, several candidate genes which were associated with risk of myocardial infarction were identified. However, the polymorphisms that were associated with a significant risk of myocardial infarction in women were not associated with a significant risk of this condition in men. The sex-based difference in the association between genetic polymorphisms and the risk of myocardial infarction might be attributable, at least in part, to the difference in estrogenic activity between men and women. The estrogenic activity is mainly regulated at three different levels affecting (i) the activity of enzymes involved in estrogen synthesis, (ii) the responsiveness to estrogens and (iii) the activity of enzymes involved in the degradation of estrogens. It has previously been demonstrated that polymorphisms in the estrogen receptor-α gene are associated with coronary artery disease, hypertension and augmented response of HDL cholesterol to hormone-replacement therapy (HRT) in postmenopausal women, supporting the notion that estrogen-related polymorphisms may modulate cardiovascular risk factors for ischemic heart disease.

The first step in the degradation of estrogens is a hydroxylation mediated by various isoforms of CYP450 enzymes. As a result several different hydroxylated estrogen metabolites, some of which are denoted CE, appear. Some of these CE possess potent biological activities. CE are at a large extent O-methylated by the COMT enzyme. The resulting methylated metabolites have no remaining estrogenic activity. The variant low activity COMT enzyme has previously been shown to have a 60–75% lower biological activity than the high activity COMT enzyme. Thus, the low activity COMT enzyme should theoretically increase the serum levels of estrogens and CE. The novel finding in the present study, that males with the low activity COMT genotype have increased serum levels of estradiol, together with the recent finding that serum estradiol levels three hours after treatment with estradiol valerate were increased in postmenopausal women with the low activity COMT genotype compared with women with the high activity COMT genotype, clearly underline the role of the COMT genotype for serum levels of estradiol. Therefore, one might speculate that future algorithms for individualizing recommendations of HRT for postmenopausal women and/or oral contraceptives might include pharmacogenomic screening of biological responsiveness, including estrogen receptor-α polymorphisms, as well as the metabolism of exogenous estrogens, including the COMT polymorphism.

The main finding, in the present study, is that patients homozygous for the low activity COMT genotype had an incidence of myocardial infarction which was lower than that of patients with the high activity genotype. In the design of the present study the cases and the controls were already matched for age, sex and smoking status. Conditional logistic regression was used to adjust for other known cardiovascular risk factors including diabetes mellitus, cholesterol and triglycerides. The effect of the polymorphism remained virtually the same after adjustment for these risk factors, suggesting that the COMT genotype is an independent risk factor for myocardial infarction.

The mechanism behind the protective effect of the low activity COMT genotype on myocardial infarction is not clear. However, one may speculate that altered estrogen status could be involved in the protective effect of this genotype since the low activity COMT genotype gives less efficient removal of estrogens, which as a consequence could lead to more cardiovascular protection from estrogens. It has also been speculated that it is not the estrogens themselves but their hydroxylated metabolites that mediate at least part of the cardiovascular protection provided by estrogen. Thus, increased levels of estrogens and/or their hydroxylated metabolites might be involved in the cardioprotective effect of the low activity COMT genotype.

In our study the cardioprotective effect of the low activity COMT genotype was most evident in older patients. The reason for this could be that at younger ages the levels of estrogens might be sufficient to provide cardiovascular protection independent of COMT activity, whereas with increasing age the levels decline and approach a critical level where the low activity COMT enzyme results in protection by slowing down the degradation of estrogens and their metabolites.

The low activity COMT genotype was associated with higher serum levels of estradiol in males but not in females in our study. The women in our study were in ages where premenopausal as well as menopausal and postmenopausal status were not available to us. Furthermore, serum levels of estradiol may vary considerably due to confounding factors, and it is not surprising that we do not find an association with COMT genotype in females. Future studies in more homogenous groups are needed to explore the association between COMT genotype and estradiol levels in women. In men on the contrary, serum levels of estradiol are not influenced by monthly fluctuations and the levels decline to a much lesser extent with age. Therefore, our cohort was more suitable for investigating the association between COMT genotype and estradiol levels in men than in women.

The COMT enzyme is also involved in the metabolism of catecholamines. Therefore, besides the effect on estrogen metabolism, it cannot be excluded that the results of our study may partly be due to effects on this system.

An important strength of the present study is that it is a prospective study with very well characterized subjects and cardiovascular endpoints. Findings from some initial retrospective studies have not been possible to confirm in later prospective studies, underlining the importance of well designed prospective studies in the investigation
of candidate genes for genetic associations to myocardial infarction.\textsuperscript{3,10} The interpretations of our findings are limited by the fact that only hypertensive patients were included. Thus, we believe that the prospective design of our study is well suited but our findings need to be confirmed in other studies that include patients from other populations and different cardiovascular risk groups.

In conclusion, our findings suggest that the low activity COMT genotype is protective against myocardial infarction. One may speculate that the altered estradiol status could be involved in the protective effect of this genotype.

Acknowledgments

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