5-Hydroxytryptamine 5-HT<sub>2A</sub> receptor and 5-hydroxytryptamine transporter polymorphisms in acute myocardial infarction

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ABSTRACT

This study was designed to analyse possible associations between DNA polymorphisms in the 5-hydroxytryptamine (5-HT; serotonin) 5-HT<sub>2A</sub> receptor and the 5-HT transporter (5-HTT) genes, and myocardial infarction (MI). 5-HT has been shown to be involved in cardiovascular pathophysiology. In addition to platelet aggregation and vascular contraction, 5-HT induces hyperplasia of artery smooth muscle cells. Recently, a 5-HT transporter gene polymorphism has been associated with MI. To determine the influence of genetic variation at the 5-HT<sub>2A</sub> receptor (T102C polymorphism) and the 5-HTT (insertion/deletion polymorphism) on the risk of developing early MI, we genotyped 210 MI patients of < 55 years old and 238 healthy control subjects for DNA polymorphisms in these genes. In addition, we genotyped 95 patients with late-onset MI (≥ 60 years old) to analyse the effects of these polymorphisms on the age at which the first MI episode occurred. The 5-HT<sub>2A</sub> receptor polymorphism was not associated with MI in our population. In addition, since the 5-HT<sub>2A</sub> receptor gene and genotype frequencies did not differ between patients with early and late onset of MI, this polymorphism does not appear to have an effect on age at the first MI episode. Gene and genotype frequencies for the 5-HTT promoter did not differ between patients < 55 years old and healthy controls (independent of smoking status). However, homozygotes for the deletion (the ss genotype, where s denotes the short allele) were present at a significantly higher frequency in patients > 60 years old compared with patients < 55 years old (P = 0.009; P = 0.004 when only smokers were compared). According to our data, the ss genotype would seem to have a protective role against MI, delaying the age of onset of the first episode, especially among smokers. This could be a consequence of the lower 5-HTT levels linked to the s allele, so that individuals homozygous for the ss genotype may have lower 5-HT re-uptake by platelets.

INTRODUCTION

5-Hydroxytryptamine (5-HT; serotonin) induces platelet aggregation, vascular contraction and hyperplasia of artery smooth muscle cells [1–3]. Platelet aggregation and vascular contraction are mediated through the binding of 5-HT to the 5-HT<sub>2A</sub> receptor [4,5]. A polymorphism in the 5-HT<sub>2A</sub> receptor gene (T102C) has been associated with MI. To determine the influence of genetic variation at the 5-HT<sub>2A</sub> receptor (T102C) and 5-HTT (insertion/deletion) polymorphisms on the risk of developing early MI, we genotyped 210 MI patients of < 55 years old and 238 healthy control subjects for DNA polymorphisms in these genes. In addition, we genotyped 95 patients with late-onset MI (≥ 60 years old) to analyse the effects of these polymorphisms on the age at which the first MI episode occurred. The 5-HT<sub>2A</sub> receptor polymorphism was not associated with MI in our population. In addition, since the 5-HT<sub>2A</sub> receptor gene and genotype frequencies did not differ between patients with early and late onset of MI, this polymorphism does not appear to have an effect on age at the first MI episode. Gene and genotype frequencies for the 5-HTT promoter did not differ between patients < 55 years old and healthy controls (independent of smoking status). However, homozygotes for the deletion (the ss genotype, where s denotes the short allele) were present at a significantly higher frequency in patients > 60 years old compared with patients < 55 years old (P = 0.009; P = 0.004 when only smokers were compared). According to our data, the ss genotype would seem to have a protective role against MI, delaying the age of onset of the first episode, especially among smokers. This could be a consequence of the lower 5-HTT levels linked to the s allele, so that individuals homozygous for the ss genotype may have lower 5-HT re-uptake by platelets.

Key words: association study, DNA polymorphism, 5-hydroxytryptamine receptor, 5-hydroxytryptamine transporter, myocardial infarction.

Abbreviations: 5-HT, 5-hydroxytryptamine (serotonin); 5-HTT, 5-hydroxytryptamine transporter; MI, myocardial infarction; l and s alleles, long and short alleles respectively of the 5-HTT insertion/deletion polymorphism; OR, odds ratio; CI, confidence interval.

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with the clinical response to 5-HT₂A receptor antagonists, suggesting that this polymorphism influences 5-HT₂A receptor function [6].

5-HT is transported by the 5-HT transporter (5-HTT), which is encoded by a polymorphic gene. One of the 5-HTT polymorphisms is located in the promoter region, approx. 1 kb upstream of the transcription initiation site. This polymorphism is a 44 bp insertion/deletion, and the deletion (long; l) allele has been associated with higher promoter activity and increased 5-HT uptake in blood platelets [7].

Some of the effects of 5-HT, such as vascular contraction and platelet aggregation, can lead to thrombus formation, and have been implicated in the pathogenesis of myocardial infarction (MI) [8]. Moreover, smoking is a well-documented risk factor for cardiovascular disease, and an increased 5-HT receptor density among smokers has been described [5]. Thus polymorphisms in the 5-HT₂A receptor and 5-HTT genes could influence the risk of developing MI [9–11]. To investigate the relationship between polymorphisms in the 5-HT₂A receptor (T102C) and 5-HTT [(l/s (long/short)] genes and MI, we genotyped a number of MI patients and compared them with healthy control subjects.

Statistical analysis
The Chi-square test was used to compare genotype and gene frequencies between the groups. The odds ratios (ORs) and their 95% confidence interval (95% CI) were also calculated. ANOVA was used to compare average values of biochemical parameters between genotypes. The effect of genotype on the age of first MI episode was analysed by logistic regression. All statistical analyses were performed using the SPSS computer program.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MI patients</th>
<th>MI patients</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>210</td>
<td>95</td>
<td>238</td>
</tr>
<tr>
<td>平均年龄</td>
<td>45±6</td>
<td>66±7</td>
<td>43±6</td>
</tr>
<tr>
<td>血压</td>
<td>74(35%)</td>
<td>39(40%)</td>
<td>36(15%)</td>
</tr>
<tr>
<td>吸烟者</td>
<td>201(96%)</td>
<td>81(85%)</td>
<td>99(42%)</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>179±21</td>
<td>141±70</td>
<td>125±82</td>
</tr>
<tr>
<td>TC (mg/dl)</td>
<td>221±55</td>
<td>199±40</td>
<td>200±41</td>
</tr>
<tr>
<td>HDL-胆固醇 (mg/dl)</td>
<td>30±9</td>
<td>31±8</td>
<td>52±14</td>
</tr>
</tbody>
</table>

Table 1 Anthropometric characteristics and average values of triacylglycerol (TG), total cholesterol (TC) and high-density lipoprotein (HDL)-cholesterol in patients and controls
Table 2 Gene and genotype frequencies for the polymorphisms in the 5-HTT and 5-HT2A genes in MI patients and healthy controls

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Genotype/gene</th>
<th>Controls (n = 238)</th>
<th>MI patients ≤ 55 years old (n = 210)</th>
<th>MI patients ≥ 60 years old (n = 95)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT2A (T102C)</td>
<td>TT</td>
<td>52 (22%)</td>
<td>40 (19%)</td>
<td>23 (24%)</td>
</tr>
<tr>
<td></td>
<td>TC</td>
<td>119 (50%)</td>
<td>107 (51%)</td>
<td>47 (51%)</td>
</tr>
<tr>
<td></td>
<td>CC</td>
<td>67 (28%)</td>
<td>63 (30%)</td>
<td>25 (26%)</td>
</tr>
<tr>
<td></td>
<td>T</td>
<td>47%</td>
<td>45%</td>
<td>49%</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>53%</td>
<td>55%</td>
<td>51%</td>
</tr>
<tr>
<td>5-HTT (l/s)</td>
<td>ll</td>
<td>72 (30%)</td>
<td>73 (35%)</td>
<td>19 (20%)</td>
</tr>
<tr>
<td></td>
<td>ls</td>
<td>114 (48%)</td>
<td>106 (50%)</td>
<td>46 (48%)</td>
</tr>
<tr>
<td></td>
<td>ss</td>
<td>52 (22%)</td>
<td>31 (15%)</td>
<td>30 (32%)</td>
</tr>
<tr>
<td></td>
<td>l</td>
<td>54%</td>
<td>60%</td>
<td>44%</td>
</tr>
<tr>
<td></td>
<td>s</td>
<td>46%</td>
<td>40%</td>
<td>56%</td>
</tr>
</tbody>
</table>

For the 5-HTT l/s polymorphism, the ll genotype was present at a significantly higher frequency in patients < 55 years old compared with patients > 60 years old (P = 0.009; OR = 2.13, 95% CI 1.18–3.78).

Biochemical values did not differ according to 5-HT2A receptor or 5-HTT genotype in patients < 55 years old, controls or patients > 60 years old. In addition, no differences were found when only patients with hypertension were compared (results not shown).

DISCUSSION

Platelet aggregation contributes to thrombus formation, which leads to episodes of MI [1]. As an inductor of platelet aggregation and vascular contraction, 5-HT has been implicated in coronary artery disease. This is also supported by the observation that inhibitors of 5-HT reuptake protect against MI [8]. In addition, 5-HT stimulates the hyperplasia of artery smooth muscle, thus contributing to the endothelial dysfunction that characterizes the pathogenesis of cardiovascular disease [2].

In the present study, we genotyped a number of MI patients and healthy control subjects for DNA polymorphisms in the genes encoding the 5-HT2A receptor and 5-HTT. Our aim was to elucidate whether the variation at these genes contributes to the risk of suffering a MI, or has any effect on the age at the first MI episode.

Gene and genotype frequencies for the 5-HTT polymorphism did not differ between patients < 55 years old and controls. When we only compared smokers, patients < 55 years old had a higher frequency of the ll genotype [36% in patients (n = 201) compared with 25% in controls (n = 99)], but the difference remained non-significant (P = 0.088).

The ll genotype was present at a significantly higher frequency in MI patients < 55 years old compared with patients > 60 years old (P = 0.009; OR = 2.13, 95% CI 1.18–3.78), suggesting an effect of this polymorphism on the age of the first MI episode. The difference was even greater when only smokers were compared [36% (n = 201) for patients < 55 years old compared with 17% for patients > 60 years old (n = 81); P = 0.004]. Patients with the ll genotype had a significantly lower age at first MI than patients with the ls or ss genotype (48.29 ± 12.08 and 54.92 ± 13.96 years respectively; P < 0.001). This difference was also found when only the patients < 55 years old were compared (ll genotype, 43 ± 6 years; ls or ss genotype, 45 ± 5 years; P = 0.017).

Biochemical values did not differ according to 5-HT2A receptor or 5-HTT genotype in patients < 55 years old, controls or patients > 60 years old. In addition, no
differ between patients with an early (< 55 years old) or late (> 60 years old) first MI episode, suggesting a lack of association with the age of onset of MI.

An association between the 5-HTT genotype and MI has been described among Japanese subjects [9]. More recently, Fumeron et al. [23] reported an increased frequency of this genotype in a case–control study based on 671 male MI patients from four European populations. In our population, the ll genotype was present at a non-significantly higher frequency among MI patients < 55 years old compared with controls, and the difference was greater when only smokers were compared. However, this genotype was at a significantly lower frequency among MI patients > 60 years old compared with those < 55 years old, and the difference was again greater when only smokers were compared. Moreover, patients with the ll genotype were significantly younger than patients with the ls or ss genotype. According to our data, the ss genotype appears to have a protective effect, delaying the onset of the first MI episode, and this effect is more relevant among smokers. It is possible that the association between the 5-HTT polymorphism and MI depends on accumulated cigarette consumption. In this case, smokers with the ll genotype would have their first MI episode at a lower accumulated cigarette consumption, showing a lower average age compared with smokers with the ls or ss genotype.

Our work has several limitations, some of which are inherent to most case–control studies. First, we analysed a limited number of patients and control subjects. However, we genotyped individuals from a homogeneous population, and the frequencies of the two polymorphisms were in Hardy–Weinberg equilibrium, suggesting that the observed frequencies represent the true gene and genotype frequencies in our population. In addition, the association between a gene variant and MI could depend on the cardiovascular risk factors in each population. Cigarette smoking is strongly associated with MI in our population, and smokers are likely to have an increased platelet 5-HT \textsubscript{2A} receptor density [5]. It is thus possible that polymorphisms in 5-HT-related genes contribute to the risk of MI in populations where smoking is the commonest risk factor, but not in populations where hypercholesterolaemia or hypertension play a more important role as risk factors for MI.

ACKNOWLEDGMENTS

This work was supported by grants FIS-01/0356 and FICYT-MED01-03 (to E.C.). P.G. and M.G.-C. are supported by predoctoral fellowships from Principado de Asturias-FICYT and Sociedad Asturiana de Cardiología respectively.

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Received 18 September 2002; 31 October 2002; accepted 5 December 2002