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Letter to the Editor

No evidence for the involvement of the lipoxin A4 receptor (FPR2/ALX) gene in the susceptibility to coronary artery disease

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The lipoxin A4 receptor (FPR2/ALX) belongs to the class A rhodopsin G protein-coupled receptor superfamily and plays a role in chemotaxis and activation of phagocytes (1). Several structurally diverse agonistic ligands, including peptides and lipid mediators have been shown to bind to the FPR2/ALX (2). Since the discovery of serum amyloid A (SAA) as a pro-inflammatory ligand for FPR2/ALX, more peptides were identified, which all mediate a pro-inflammatory activation of leukocytes through FPR2/ALX (1). In contrast, FPR2/ALX can also bind anti-inflammatory ligands, such as lipoxin A4 (LXA4) and annexin-1 (2). Indeed, LXA4 was shown to inhibit neutrophil migration, to induce chemotaxis of monocytes and to promote the non-phlogistic phagocytosis of apoptotic neutrophils by macrophages (2). Thus, FPR2/ALX might induce anti-inflammatory effects when pro-resolving mediators are present.

Expression of the FPR2/ALX has been observed in several human chronic inflammatory diseases, such as dermatitis, rheumatoid arthritis, and severe asthma, however, the role of the FPR2/ALX in atherosclerosis has not been investigated (3).

Atherosclerosis is a multifactorial disease that is characterized by chronic inflammation at every stage with leukocytes infiltrating the arterial intima (4). To investigate the role of FPR2/ALX in atherosclerosis we screened the FPR2/ALX gene for polymorphisms and analyzed whether the detected polymorphisms are associated with coronary artery disease (CAD) in a case-control study involving 497 Caucasians (5, 6). We initially screened the promoter, the coding region and the 3′ region of the FPR2/ALX gene of 98 Caucasians for polymorphisms by DHPLC and detected eight variations, five of which were located in the promoter region, one in the coding region, and two in the 3′ region (Table 1). Seven of these variations proved to be polymorphisms with minor allele frequencies between 5% and 32%, while variant c.410G>T was a private mutation. The genotype frequency for all polymorphisms was in agreement with those predicted by the Hardy-Weinberg equilibrium in the case-control sample.

This case-control sample for CAD included 259 patients with >50% stenosis in at least one coronary artery and 238 subjects without a history of CAD, stroke, or peripheral vascular disease (5, 6). Written informed consent was obtained from all participants and the Local Ethics Committee approved the study. The case-control study has a power of 25%–80% to detect an OR of 1.7 for minor allele frequencies of 5%–30%.

Logistic regression analysis revealed no association between five polymorphisms of the FPR2/ALX gene and CAD (Table 1), while the T allele of the c.-7893C>T polymorphism was associated with an increased risk for CAD (OR=2.9; confidence interval 1.2–7.1; p-value: 0.018). This c.-7893C>T polymorphism is located in the core promoter of the FPR2/ALX gene and could therefore modify the binding site for a transcription factor and hence alter transcription of the gene (8).

To test whether the C>T transition affects the transcriptional activity of the promoter, we subcloned the FPR2/ALX core promoter upstream of a luciferase reporter gene and introduced all the variations of the minor alleles of the three promoter polymorphisms into individual constructs. These constructs were then transiently transfected into THP-1 macrophages and the transcriptional activity of the different promoter alleles was judged from the intensity of the luciferase reporter enzyme (Figure 1, dual-luciferase reporter assay). None of the three polymorphisms c.-7978A>G, c.-7986A>G and c.-7893C>T altered the transcriptional activity of the FPR2/ALX promoter in macrophages, suggesting that the observed association of the SNP c.-7893C>T with CAD was rather a chance finding than a real association. This is
Table 1 Logistic regression model predicting CAD status for cases.

<table>
<thead>
<tr>
<th>NM01005738</th>
<th>Allele frequency, %</th>
<th>Genotype controls, %</th>
<th>Genotype cases, %</th>
<th>OR</th>
<th>95% CI</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.8751A&gt;G</td>
<td>A: 68.6</td>
<td>G: 31.4</td>
<td>47.3/43.9/8.9</td>
<td>47.5/41.2/11.3</td>
<td>1.72</td>
<td>0.75–3.94</td>
</tr>
<tr>
<td>rs11666254</td>
<td>T: 68.3</td>
<td>G: 31.7</td>
<td>46.4/43.9/9.7</td>
<td>47.5/41.6/10.9</td>
<td>0.60</td>
<td>0.26–1.36</td>
</tr>
<tr>
<td>c.7968A&gt;G</td>
<td>A: 73</td>
<td>G: 27</td>
<td>49.8/46/4.2</td>
<td>52.1/42/5.8</td>
<td>0.98</td>
<td>0.69–1.40</td>
</tr>
<tr>
<td>rs35215887</td>
<td>T: 95</td>
<td>G: 5</td>
<td>92.4/7.6/0</td>
<td>89.1/9.7/1.2</td>
<td>1.42</td>
<td>0.75–2.68</td>
</tr>
<tr>
<td>c.7893C&gt;T</td>
<td>C: 88.2</td>
<td>T: 11.8</td>
<td>77.2/22.8/0</td>
<td>77.4/20.6/1.9</td>
<td>2.93</td>
<td>1.20–7.13</td>
</tr>
<tr>
<td>rs56238033</td>
<td>Private mutation</td>
<td></td>
<td></td>
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</tbody>
</table>

DNA extracted from EDTA-supplemented blood was genotyped by tetra-primer analysis for each variation (7). Association with CAD was analyzed by logistic regression using an additive genetic model adjusted for age and sex. p < 0.05.

We observed six haplotypes with a frequency >1% which summed up to 94% of all haplotypes in the sample.

In conclusion, the FPR2/ALX gene was not associated with CAD in our small case-control study, however, our study suggests that the FPR2/ALX gene is conserved in humans, since no polymorphism in the coding region and no functional polymorphisms in the core promoter region have been identified.

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References