

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>A 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

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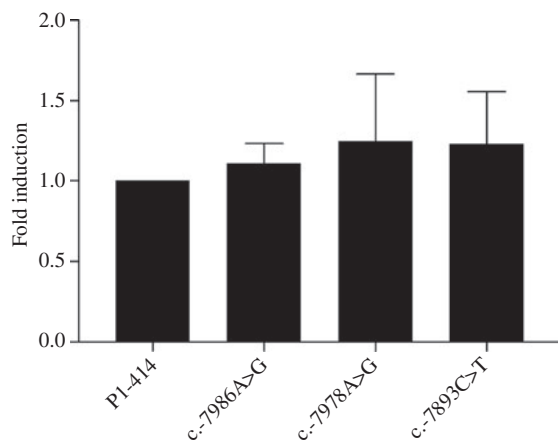
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Table 1 Logistic regression model predicting CAD status for cases.

NM01005738	Allele frequency, %	Genotype controls, %	Genotype cases, %	OR	95% CI	p-Value
c.-8751A>G rs11666254	A: 68.6 G: 31.4	47.3/43.9/8.9	47.5/41.2/11.3	1.72	0.75–3.94	0.20
c.-8461T>G rs11670660	T: 68.3 G: 31.7	46.4/43.9/9.7	47.5/41.6/10.9	0.60	0.26–1.36	0.22
c.-7986A>G rs35215887	A: 73 G: 27	49.8/46/4.2	52.1/42/5.8	0.98	0.69–1.40	0.92
c.-7978A>G rs35225016	A:95 G: 5	92.4/7.6/0	89.1/9.7/1.2	1.42	0.75–2.68	0.29
c.-7893C>T rs56238033	C: 88.2 T: 11.8	77.2/22.8/0	77.4/20.6/1.9	2.93	1.20–7.13	0.02
c.410G>A c.1315A>G rs17695052	A: 89.1 G: 10.9	76.8/23.2/0	Private mutation 80.5/18.3/1.2	0.66	0.19–2.28	0.51
c.1346C>T rs17695064	C: 89.4 T: 10.6	77.2/22.4/0.4	81.3/17.9/0.8	0.51	0.17–1.58	0.24

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. $p < 0.05$.

supported by the skewed distribution of the homozygous TT genotypes between cases and controls in the study sample. While the TT genotype was observed to a low frequency in cases, this genotype was absent in controls (1.95% vs. 0%, respectively). The *FPR2/ALX* haplotypes derived from the seven polymorphisms also showed no association with CAD.

**Figure 1** Basal transcription of the different *FPR2/ALX* core promoter alleles observed.

Luciferase expression of the four *FPR2/ALX* core promoter alleles was determined (c.-7986A>G: A-to-G base exchange at position -7986; c.-7978A>G: A-to-G base exchange at position -7978; c.-7893C>T: C-to-T base exchange at position -7893). Human THP-1 monocytes were transfected with the constructs mentioned and a renilla control construct, differentiated into macrophages and the luciferase activity was measured and normalized against the activity of the renilla luciferase 48 h later. Results were calculated as fold induction over the *FPR2/ALX* wild-type core promoter. All experiments were done in triplicate and bars indicate the means of three independent experiments with three plasmid preparations. Values represent fold induction compared to the P1-414 vector and the error bars represent the standard deviation (\pm SD).

We observed six haplotypes with a frequency >1% which summed up to 94% of all haplotypes in the sample (data not shown).

In this study, we identified seven polymorphisms in the promoter and in the 3' region of the *FPR2/ALX* gene, but no polymorphism was detected in the coding region of the gene. Such a lack of polymorphisms in the coding region of the *FPR2/ALX* gene has previously been observed in a small study comparing the number of polymorphisms in the coding region between the *FPR2/ALX* and the closely related *FPR1* gene (9). This study found that the two genes originated from a common gene but that they had undergone markedly different evolutionary events since then. While seven polymorphisms have been detected in the coding region of the *FPR1* gene in this small Caucasian, Black and Asian sample, no polymorphism was detected in the *FPR2/ALX* coding region in the same racial groups (9). Our study supports and expands these findings, because we screened an additional 196 Caucasian alleles and did not find a polymorphism in the coding region of the *FPR2/ALX*. Such an absence of functional polymorphisms in the coding region and the lack of functional polymorphisms in the core promoter region suggest that the *FPR2/ALX* gene is highly conserved in humans and that it may be important for the correct function of leukocytes in innate immunity.

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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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research support played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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