Thrombosis Research 127 (2011) 600–602

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

Letter to the Editors-in-Chief

A common promoter variant of the gene encoding cyclooxygenase-1 (*PTGS1*) is related to decreased incidence of myocardial infarction in patients with coronary artery disease

Dear Editors,

Cyclooxigenase (COX)-1, formally known as prostanglandin endo-peroxide H synthetase-1, mediates synthesis of prostaglandin H_2, which is subsequently converted to various biologically active metabolites including thromboxane (TX) A_2 [1]. TXA_2 is synthesized and released by activated platelets and strongly reinforces thrombus formation, a critical pathway in the pathogenesis of myocardial infarction (MI). Inhibition of COX-1-derived TXA_2 in platelets by low-dose aspirin administration reduces incidence of MI [2]. Hypothetically, MI-risk could also be modified by genetic variants that affect activity or expression of COX-1. Many single nucleotide polymorphisms (SNP) in the gene encoding COX-1 (*PTGS1*) have been described; including functional alterations in both coding and non-coding regions [3,4]. We have evaluated if two of such variations are related to the risk of MI in a historic cohort of patients with coronary artery disease (CAD).

Patients were enrolled from a larger cohort of Caucasians who underwent scheduled coronary angiography or percutaneous trans-luminal coronary angioplasty with implantation of drug eluting stent at the Latvian Centre of Cardiology. The inclusion criteria were CAD confirmed with digital angiography, defined as coronary artery stenosis >=50%, and available information regarding previous MI. The definition of previous MI was: elevated myocardial damage markers (troponin I or T, or creatinine kinase MB fraction) during index hospitalization as indicated in discharge letters or, if discharge letters were not available, pathologic Q wave in at least two contiguous leads. Patients with dubious history of MI were excluded from analysis.

Data on conventional cardiovascular risk factors (age, gender, smoking status, diabetes, arterial hypertension, body mass index, and total cholesterol) were also recorded. The study was approved by the Central Medical Ethics Committee of Latvia, and written consent was obtained from each participant.

A promoter polymorphism (-707 A>G; rs10306114) and a nonsynonymous coding variation (10742 C>A; rs5789) were chosen for analysis. The selection was based on the reported functional impact and presence in Caucasians [3–5]. The -707 A>G is one of seven SNPs (-1749 T>C, -1598 G>A, -1202 A>G, -1201 A>G, -1006 G>A, -918 A>G, and -707 A>G) within the promoter region of *PTGS1* that are in nearly complete linkage disequilibrium (LD) [3]. The minor haplotype has been associated with low urinary 11-TXB2 levels [5]. In Caucasians it is also present at an appreciable frequency of 6-8% and in strong LD with nonsynonymous coding 278 C>T (P17L; rs3842788) polymorphism of exon 2 [3]. The 10742 C>A variation is located in exon 7 and causes L237M substitution. This replacement was predicted to influence enzyme function through its effect on dimerization [3,4] and led to a decreased COX-1 activity *in vitro* [3]. In addition, leucine 237 is conserved across species, which further supports a functional significance of the position [3]. The reported minor allele frequency is 3-4% in Caucasians [3,4]. Apart from L237M and P17L exchanges, few other *PTGS1* missense variants have been detected but those are either absent in Caucasians or seem functionally irrelevant.

Genomic DNA isolated from fresh blood by standard phenol extraction was provided by the Latvian Genome centre. PCR primers GGAAACTGAGGGAGATGAC (forward) and GGTCTAACGCACTCTAC (reverse) were used to amplify a 669 bp fragment encompassing the promoter polymorphism, whereas TTCACCCACCAGTTCTTC (forward) and GAGCTCCAAAATCCACCT (reverse) were used for amplification of a 493 bp region surrounding the missense variation. Genotyping was then performed by matrix-assisted laser desorbtion/ionization time-of-flight mass spectrometry method employing the ddNTP extension protocol as provided by manufacturer (Bruker Daltonics). 5′-biotinilated primers TGAGCACCTACLACATGCTGG and GCAGTTGATACTGALGCTCCA were used to detect -707 A>G and 10742 C>A alleles, respectively.

Statistical analysis was performed with Fisher's exact test and odds ratio (OR) was calculated. Adjustment for conventional cardiovascular-risk factors was done with logistic regression. Results were considered to be significant at 2-tailed values of $p<0.05$.

Medical and genetic characteristics of patients are summarized in Table 1. Majority of them (67.4%, n=617) had previous myocardial infarction, while minority (38.3%, n=351) had experienced a premature MI, defined as MI before age of 55 years in men and 65 years in women. Both polymorphisms were present in the study population with allelic distribution being in Hardy-Weinberg equilibrium ($p=0.185$ for -707 A>G and $p=0.700$ for 10742 C>A). Minor promoter variant, the -707G allele, had prevalence typical for other Caucasians [3,5]. The

Table 1
Characteristics of the study population (n=916).

Myocardial infarction (MI)		
	History of MI, n (%)	617 (67.4)
	Premature MI, n (%)	351 (38.3)
Cardiovascular risk factors		
	Age mean, years (SD)	58.4 (9.6)
	Male, n (%)	695 (75.9)
	Current or former smoker, n (%)	570 (62.2)
	Hypertension, n (%)	594 (64.8)
	Diabetes mellitus, n (%)	122 (13.3)
	BMI mean, kg/m^2 (SD)	28.6 (4.4)
	Total cholesterol, mmol/l (SD)	5.3 (1.4)
Alleles and genotypes, n (%)		
A>G	A	1723 (94.1)
	G	109 (5.9)
	A/A	808 (88.2)
	A/G	107 (11.7)
	G/G	1 (0.1)
C>A	C	1809 (98.7)
	A	23 (1.3)
	C/C	893 (97.5)
	C/A	23 (2.5)

0049-3848/$ – see front matter
doi:10.1016/j.thromres.2010.12.019

Table 2
Prevalence of genotypes according to history of myocardial infarction (MI).

Characteristic		A>G genotype, n (%)		P	C>A genotype, n (%)		P
		A/A	A/G + G/G		C/C	C/A	
History of MI	Yes	558 (90.4)	59 (9.6)	0.004 (0.010*)	600 (97.2)	17 (2.8)	0.654 (0.607*)
	No	250 (83.6)	49 (16.4)		293 (98.0)	6 (2.0)	
Premature MI	Yes	322 (91.7)	29 (8.3)	0.009 (0.014*)	342 (97.4)	9 (2.6)	1.000 (0.697*)
	No	486 (86.0)	79 (14.0)		951 (97.5)	14 (2.5)	

*After adjustment for age, gender, smoking, hypertension, diabetes, body mass index, and total cholesterol.

frequency of missense variant, the 10742A allele, was, however, relatively low and constituted only 1.3%.

Main statistical results are presented in Table 2. Distribution of the promoter variants by the history of myocardial infarction strongly deviated from proportional. First, carriers of the -707G allele (G/G + A/G genotypes) were substantially less frequent among cases of MI (9.6%; n = 59) than among patients without a previous MI (16.4%; n = 49; OR = 0.584, 95% CI = 0.410-0.830, p = 0.004). This association retained significance after adjustment for conventional cardiovascular- risk factors (p = 0.010). Furthermore, carriers of the minor variant were also rare among patients who had experienced a premature MI as compared to other participants (8.3% versus 14.0%; OR = 0.591, 95% CI = 0.394-0.885; p = 0.009 and p = 0.014 before and after adjustment, respectively). The difference of distribution of the -707G allele by history of MI was not gender-specific. In men, carriers of this variant constituted only 9.5% (n = 45) of MI-cases compared to 16.0% (n = 35) of patients without MI. In women, the corresponding numbers were 9.9% (n = 14) and 17.5% (n = 14). Similar result was obtained with regard to premature MI. No association with myocardial infarction was detected for the coding 10742 C>A (L237M) substitution.

Our analysis demonstrated that the -707G allele, a constituent of a common minor *PTGS1* promoter haplotype, was significantly related to decreased incidence of myocardial infarction among patients with CAD (OR = 0.584, p = 0.004). The same held true with respect to premature MI (OR = 0.591, p = 0.009). A rationale for this protective genetic effect could be provided by results of two large studies [3,5]. First, sequence analysis has suggested that several variant alleles present on the haplotype may disrupt putative transcription factor binding [3], thus possibly resulting in decreased transcription and levels of COX-1. Second, in line with the above, carriers of the variant haplotype were found to have significantly lower urinary 11-dehydro-TXB2 levels than non-carriers, which is a strong indication of a low COX-1 metabolic activity [5]. Decreased platelet TXA2 production would be predicted to reduce platelet aggregation and, consequently, the risk of myocardial infarction. We found, that in Latvian CAD patients the minor promoter variant lowered odds of MI nearly twice. Of note, the same COX-1 haplotype was recently reported to increase the risk of bleeding complications in patients undergoing elective coronary angiography [6], which is consistent with anti-thrombotic properties of this genetic variant.

An alternative explanation cannot be excluded. In Caucasians there is a substantial LD between promoter haplotype and nonsynonymous 278 C>T (P17L) coding SNP. It may therefore be possible that the associations we detected merely reflect an influence of the missense variant. However, it is difficult to attribute a molecular basis to putative effect of P17L substitution as it is located in signal peptide not mature cyclooxygenase. Also, *in vitro* this exchange did not alter metabolic activity of the enzyme [3].

Possible relation of *PTGS1* promoter variation to myocardial infarction has already been assessed by others, yet no association with MI [5] or premature MI [7] was detected. Of note, COX-1 through its multiple effects may influence not only pathogenesis of myocardial infarction but also progression of atherosclerosis. Therefore, the discrepancy between results of our and aforementioned analysis could reside in the principally different study designs. Instead of using the case/healthy control approach, we have focused on patients with CAD confirmed by angiography.

A limitation of our retrospective study was lack of exact information on previous treatment with aspirin at the time of MI. Since the analysed haplotype may influence response to aspirin [8], we can only speculate whether -707G variant affects natural platelet activity or modify effect of aspirin, or both. It should be noted also that survival and selection biases cannot be excluded in cases and controls, respectively. As controls we selected patients with no history of MI, but with coronary stenosis ≥50% in order to focus only on evidence of thrombotic event in patients with established CAD.

Another variation we analysed, the 10742 C>A (L237M), showed no relation to myocardial infarction although there is a strong experimental evidence for its functional impact on COX-1 activity [3]. Yet, carriers of the missense allele were very few, which may have limited the power of our analysis to detect an association, should such exist.

In conclusion, our results suggest that *PTGS1* promoter haplotype bearing the -707G allele may decrease the risk of myocardial infarction in patients with coronary artery disease.

Conflict of interest statement

No conflict of interest.

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17 June 2010