ORIGINAL ARTICLE

Functional promoter polymorphism of cyclooxygenase-2 modulates the inflammatory response in stable coronary heart disease

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KEY WORDS

coronary heart disease, cyclooxygenase-2, inflammatory response, promoter polymorphism, prostaglandin E_2

ABSTRACT

INTRODUCTION Inflammatory mediators, including prostanoids produced by inducible cyclooxygenase-2 (COX-2), play a significant role in the development of atherosclerosis. A regulatory region of COX-2 gene has a common $_{-765}$G>C polymorphism. Functional effects of this polymorphism and its association with atherosclerosis phenotypes have not been fully understood.

OBJECTIVES The aim of the study was to evaluate the association between COX-2 $_{-765}$G>C polymorphism and the inflammatory response in patients with stable CAD.

PATIENTS AND METHODS We studied systemic prostaglandin E_2 (PGE_2) metabolism, the levels of soluble CD163 (sCD163) in serum (a marker of monocyte/macrophage activation), and COX-2 $_{-765}$G>C polymorphism in patients with stable CAD. We also tested the patients for functional effects of COX-2 $_{-765}$G>C polymorphism using cell lines, using the constructs in which red fluorescent protein expression was controlled by a large segment of COX-2 regulatory region.

RESULTS Patients with stable CAD carrying the variant allele $_{-765}$C allele had increased urinary excretion of PGE_2 metabolite and higher serum levels of sCD163 than patients carrying the $_{-765}$G allele. In contrast to these clinical findings, in vitro functional studies demonstrated that the $_{-765}$C variant allele was less responsive than $_{-765}$G allele to a wide range of COX-2 inducers.

CONCLUSIONS A substantial part of total PGE_2 biosynthesis is contributed by activated monocytes/macrophages in stable CAD. The exact mechanism of activation of this pathway in CAD requires further research because of the conflicting results on COX-2 $_{-765}$G>C polymorphism provided by clinical studies and in vitro functional studies.

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INTRODUCTION Atherosclerosis is an inflammatory disease,[1] and inflammatory mediators (including prostanoids) seem to play a key role in its pathogenesis. Prostaglandins (PGs) are derived from arachidonic acid via PG endoperoxide G/H synthase, commonly known as cyclooxygenase (COX), which first converts the arachidonic acid into PG endoperoxide intermediates, PGG_2 and PGH_2. These, in turn, are further metabolized by isomerases and synthases to form PGs and thromboxane A_2. In the arterial wall, endothelial cells are the major source of prostacyclin (PGI_2), while infiltrating monocytes/macrophages generate PGE_2. In humans, prostacyclin is a potent vasodilator and platelet inhibitor,[2,3] and in general, it acts as a restraint on endogenous stimuli to platelet activation, vascular proliferation, hypertension, and atherogenesis. The role of PGE_2 is more complex and its diverse effects may be mediated by 4 prostanoid receptors (EP1–4).[4]

There are at least 2 COX genes, encoding 2 different enzymes: COX-1 and COX-2. COX-2, a dominant source of PGI_2 and PGE_2, is upregulated in human atherosclerotic plaque ex vivo.[5]

TABLE Demographic, clinical, and genetic characteristics of patients with stable coronary artery disease in relation to sex and allele C presence

Parameter	Women (n = 37)		Men (n = 86)	
	allele C (+) n = 13	allele C (–) n = 24	allele C (+) n = 30	allele C (–) n = 56
age, years[a]	69.6 ± 9.5	62.3 ±11.4	60.9 ±11.1	62 ±11.1
BMI, kg/m^2[a]	27.4 ± 4.9	28.5 ±5.5	27.7 ±3.5	27.3 ±3.6
type 2 diabetes, n (%)	2 (15.4)	3 (12.5)	6 (20)	7 (12.5)
hypertension, n (%)	12 (92.3)	19 (79.2)	28 (93.3)	46 (82.1)
total cholesterol, mmol/l[a]	5.06 ±1.39	5.04 ±1.11	4.73 ±0.96	4.82 ±1.24
LDL cholesterol, mmol/l[a]	3.21 ±1.24	3.03 ±1.04	3.00 ±0.84	3.06 ±0.97
HDL cholesterol, mmol/l[a]	1.2 ±0.22	1.33 ±0.28	1.06 ±0.21	1.01 ±0.23
triglycerides, mmol/l[a]	1.44 ±0.59	1.19 ±0.57	1.73 ±0.88	1.65 ±0.98
plasma fibrinogen, g/l[a]	3.75 ±0.91	3.45 ±0.72	3.35 ±0.88	3.41 ±0.98
cigarette smoking, n (%)	0	4 (16.7)	7 (23.3)	9 (16.1)
age at CAD onset, years[a]	59.5 ±12.6	57 ±11.2	53.5 ±10.8	55.6 ±11
prior myocardial infarction, n (%)	4 (30.8)	11 (45.8)	13 (43.3)	25 (44.6)
history of PCIs, n (%)	3 (23.1)	4 (16.7)	7 (23.3)	14 (25)
prior stroke/TIA, n (%)	0	0	2 (6.7)	6 (10.7)
symptomatic PAD, n (%)	1 (7)	2 (8.3)	3 (10)	5 (8.9)

a mean values ± standard deviation

Abbreviations: BMI – body mass index, CAD – coronary artery disease, HDL – high-density lipoprotein, LDL – low-density lipoprotein, PAD – peripheral arterial disease, PCI – percutaneous coronary intervention, TIA – transient ischemic attack

The *COX-2* gene shows a marked variability, and more than 170 single nucleotide polymorphisms (SNPs) were listed in the NCBI/SNP database (National Center for Biotechnology Information SNP database). So far, a functional significance has been reported only for a minority of these SNPs. A variant in the *COX-2* promoter, $_{-765}$G>C (rs20 417), has been particularly interesting. It is located within a putative binding site for Spl, considered to be a positive activator of *COX-2* transcription.[6] Variable pharmacodynamic responses of this functional polymorphism were seen in COX-2 expression pattern and after ex vivo stimulation with lipopolysaccharide (LPS).[7-9] A comparison of *COX-2* promoter constructs revealed that the $_{-765}$C allele had a lower activity than $_{-765}$G allele when transfected into human cervical epithelium cancer (HeLa) cells,[6] but opposite results were obtained when transfection was conducted on human neural cells.[7] Patients carrying $_{-765}$C allele were reported to have a lower risk of myocardial infarction and ischemic stroke.[8,9] However, these clinical findings have not been confirmed by other studies.[10,11]

We hypothesized that in patients with coronary heart disease the $_{-765}$G>C *COX-2* polymorphism might influence the capacity of activated monocytes/macrophages to synthesize PGE_2, and this would be reflected by systemic PGE_2 production and associated with a specific marker of monocyte activation. We also designed experiments to directly determine the activity of the 2 promoter variants, using fluorescent protein gene expression in the transfected cells.

PATIENTS AND METHODS

Subjects A prospective, observational, cross-sectional study started on January 1, 2005 and was completed on December 31, 2006. A total of 1386 patients from southern Poland, with a suspicion of coronary artery disease (CAD), underwent invasive evaluation. Out of 974 subjects with angiographically proven CAD (coronary stenosis >70% in diameter), stable angina pectoris was diagnosed in 628 (64.5%) subjects. Of these, we recruited 274 individuals, who had regularly received aspirin at a dose of 150 mg/d, for at least 7 days before coronary angiography. The remaining 354 patients took aspirin irregularly, or at doses other than 150 mg/d, or did not receive aspirin. In the group of 274 patients, the following exclusion criteria were used: acute inflammatory and/or infectious diseases (12 patients), chronic inflammatory diseases other than atherothrombosis and/or chronic use of nonsteroidal anti-inflammatory drugs other than aspirin (49 patients), bronchial asthma (9 patients), autoimmune disorders or malignancies (3 patients), therapy with other drugs that affect hemostasis (29 patients), renal or liver insufficiency (13 patients), refusal to give consent (25 patients), disregard for appointments (lack of cooperation) (7 patients), coexisting acute psychosis (1 patient), alcoholism, or drug abuse (3 patients).

The study population ultimately comprised 123 white patients (37 females, 86 males), aged 39 to 86 years (mean 62.6 ±11.2). Their demographic, clinical, and genetic characteristics are presented in the TABLE. They were on chronic therapy with aspirin (100%), statins (88%), angiotensin-converting enzyme inhibitors or angiotensin receptor blockers (87%), β blockers (78%), nitrates (69%), calcium antagonists (48%), and diuretics (42%). The control group comprised 128 healthy subjects (59 women, 69 men), aged 24 to 70 years (mean age 41.5 ±15.8 years). These

subjects were randomly sampled from the registers provided by the local authorities of Kraków.

All patients gave written informed consent to participate in the study. The study protocol complied with the Helsinki Declaration and was approved by the University Ethical Committee.

Genotyping Common *COX2* $_{-765}$G>C polymorphism was genotyped using genomic DNA, isolated from peripheral blood as described previously.[12]

Measurement of urinary tetranor-PGE-M and 6-keto-PGF$_{1\alpha}$ Urinary concentration of 9,15--dioxo-11α-hydroxy-2,3,4,5-tetranor-prostane--1,20-dioic acid (tetranor-PGE-M) was measured by gas chromatography/mass spectrometry using internal deuterated standard.[13] Urinary tetranor-PGE-M was expressed as nanograms per mg of urinary creatinine, after recalculation for a deuterated compound to compensate the loss during preparation. Urinary level of 6-keto-PGF$_{1\alpha}$, one of the prostacyclin end metabolites, was measured in duplicate using a commercial enzyme-linked immunoassay (6-keto-PGF$_{1\alpha}$ ELISA, Cayman Chemical Co., Ann Arbor, United States) and expressed as nanograms per mg of urinary creatinine.

Measurement of serum CD163 concentration The serum levels of soluble CD163 (sCD163) antigen were measured in duplicate using a commercial ELISA (Cedarlane Laboratories, Hornby, Canada) in 44 patients with stable CAD matched for sex and age, but contrasted for $_{-765}$G>C polymorphism of *COX-2* gene. Serum samples were diluted 1:200 and processed according to the manufacturer's instructions. The intra-assay coefficient of variation was 8%.

COX-2 gene promoter expression vectors A 5' untranslated region of *COX-2* gene was amplified using primers: ct cga GGT GAG CAC TAC CCA TGA TAG A and gga tcc GCT GTC TGA GGG CGT CTG, flanking 1340 base pairs upstream of the first codon of the gene. Adapter sequences were added, recognized by uniquely cutting restriction endonucleases *Xho*I and BamHI for further cloning. Amplification products were verified by sequencing. Test plasmids were obtained by ligation of amplified allelic variants $_{-765}$G and $_{-765}$C of the *COX-2* gene to the red fluorescence protein (RFP) expression vector pDsRed2 (Clontech, Mountain View, United States). A reference expression plasmid for transient transfection experiments was the green fluorescence protein (GFP) plasmid with the cytomegalovirus promoter CMV-GFP (Clontech).

Established cell lines were tested for transfection with each variant of COX-2 gene expression vector. These were HeLa cells, human microvasculature endothelial cells (HMEC-1), human monocytes--macrophage leukemia cells (MonoMac), and human promyelocyte leukemia cells (THP-1). Transfections were performed on 3–4 million cells using electroporation procedure (BioRad gene pulser, Carlsbad, United States). The next day, regular medium was restituted and 500,000 cells were aliquoted into 6-well plate for stimulation experiments. Transformed cells were studied 48 h after transfection experiments using Coulter Epics XL flow cytometer. Stably transfected cells were selected using geneticin (G-418, Sigma, Saint Louis, United States) at a concentration of 1 mg/ml. In these experiments no GFP marker cotransfection was used, and red-channel fluorescence was compared with mock transfected cells in order to compensate for autofluorescence bias.

Several inducers of COX-2 expression were tested: bacterial LPS (Sigma, Saint Louis, United States, 0.1 μg/ml), interleukin 1β (IL-1β) (R&D Systems, Minneapolis, United States, 10 ng/ml), estradiol (Sigma, 10 nM and 100 nM), 22(R)--hydroxycholesterol (0.2 and 20 μM) and T0 901 317 (oxysterol agonist 0.25 and 2.5 μM, Cayman Chemical Co.).

Statistical analysis Urinary excretion of tetranor-PGE-M, 6-keto-PGF$_{1\alpha}$ and serum levels of sCD163 were reported as arithmetic mean with 95% confidence interval (CI). Statistical tests were performed on log-transformed data to stabilize the variance. Comparisons between genotype classes of COX-2 promoter polymorphism were performed using ANOVA. Analyses of the mean fluorescence intensity were made using geometric means of relative fluorescence units and repeated measurement ANOVA tests, with post-hoc comparisons. Cytofluorimetric data on stable transfected adherent THP-1 cells were compared using a mock transfected reference sample and expressed also as percentage of positive cells. P <0.05 was considered statistically significant.

RESULTS **Genotyping** A rare $_{-765}$C variant COX-2 allele was found in 2 homozygous (1.6%) and 41 heterozygous (33.3%) CAD patients, the remaining 80 patients (65%) were homozygous for the wild type G allele. Genotype frequencies of $_{-765}$G>C polymorphism are summarized in the **TABLE**. The minor allele frequency of this polymorphism was 18.29% and the genotypes were in Hardy-Weinberg equilibrium. No association was found between the genotypes and coronary vessel involvement as evaluated by coronary angiography (data not shown).

In the control group, the $_{-765}$C allele was found in 4 homozygous (3.1%) and 33 heterozygous (25.8%) subjects; the remaining 91 subjects (71.1%) were homozygous for the G allele. Minor allele frequency was 16.01% and the genotypes were in Hardy-Weinberg equilibrium. The distribution of genotypes in the 2 studied groups did not differ significantly.

Inflammatory biomarkers The results of inflammatory biomarkers measurements are presented

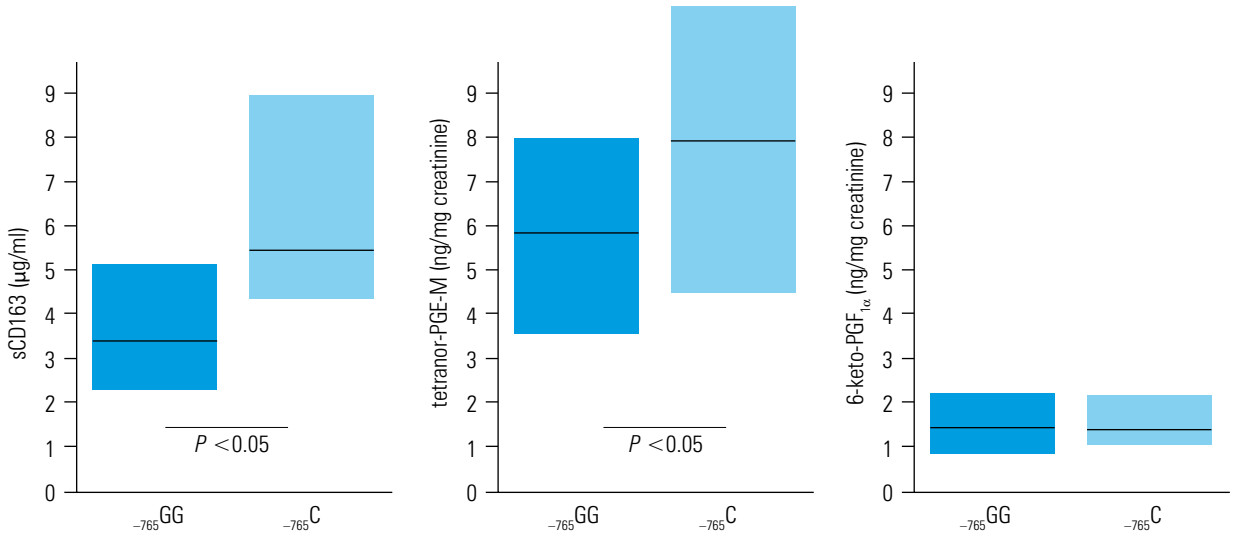

FIGURE 1 Biomarkers of inflammation in the subgroups with different COX-2 $_{-765}$G>C genotypes. Medians and 25% to 75% centiles are plotted for serum sCD163, urinary tetranor-PGE-M and urinary 6-keto-PGF$_{1\alpha}$. Genotype classes are $_{-765}$GG and $_{-765}$GC or $_{-765}$CC.

in FIGURE 1. The range of urinary tetranor-PGE-M excretion in CAD patients was 0.15 to 61.16 ng/mg creatinine with the mean 8.19 (6.42–9.96, 95% CI). An increased excretion of urinary tetranor-PGE-M was found in CAD patients carrying $_{-765}$C variant of the *COX-2* gene in comparison to GG homozygotes (8.27 vs. 5.55 ng/mg creatinine, $P = 0.03$). The range of urinary 6-keto-PGF$_{1\alpha}$ excretion in CAD patients was 0.43 to 16.3 ng/mg creatinine, with the mean 1.47 (1.32–1.67, 95% CI). There was no difference in urinary 6-keto-PGF$_{1\alpha}$ between the carriers of $_{-765}$C variant of the *COX-2* gene and GG homozygotes (1.47 vs. 1.45 ng/mg creatinine, $P = 0.9$). No correlation was found between urinary tetranor-PGE-M and urinary 6-keto-PGF$_{1\alpha}$ (Kendal tau = 0.127, nonsignificant).

The range of sCD163 levels varied from 0.63 to 14.4 µg/ml with the mean 4.59 (3.35–5.28, 95% CI). In patients carrying $_{-765}$C allele of *COX-2* gene, serum levels of sCD163 were higher than in GG homozygotes (5.41 vs. 3.37, $P = 0.023$). Several clinical parameters, i.e., age, total white cell blood count, monocyte and neutrophil count, total serum cholesterol, low- or high-density lipoprotein cholesterol, and C-reactive protein (CRP) were tested as covariant to the ANOVA model based on genotype classes. Only total serum cholesterol significantly contributed to the model ($P = 0.045$) and improved its goodness of fit ($P = 0.015$). Serum sCD163 correlated with urinary tetranor-PG-M (least median of squares robust regression $R^2 = 0.79$).

In vitro studies Using each investigated cell line, a similar regulatory property of allelic variants of $_{-765}$G>C polymorphism was observed (FIGURE 2). It consisted in the higher level of transgene expression for the wild type $_{-765}$G allele, by 49.0 ±25.1% on average. The highest overexpression was noted in THP-1 cells growing in suspension (monocyte model – 80.1%), the lowest was in the same cells but growing adherently (macrophage model – 14.5%).

These differences were significant for THP-1 cells cultured in suspension, the same cells differentiated into adherent macrophages and endothelial HMEC-1, which had the highest expression of the transgene. Due to a substantial variance of expression between replicates (n = 5), MonoMac and HeLa cells showed only a trend for overexpression of the $_{-765}$G variant. When stimulated with bacterial LPS, THP-1 cell suspension induced *COX-2* transgene of G allele by 16.1% and by 44.1% for C allele. Thus, the transgene inducibility was higher in inflammatory conditions in the variant $_{-765}$C allele.

IL-1β was a poor inducer of *COX-2* in nonadherent THP-1 cells. There were no significant differences between stimulated and resting cells, despite a high number of replicates (n = 8). Using THP-1 adherent cells, IL-1β induced the transgene in G allele transfected cells (n = 5). This result was confirmed in transiently transfected HMEC-1 cells stimulated with IL-1β. The tested cell lines were refractory to estradiol.

Adherent THP-1 cells stably transfected with *COX-2* transgenes showed analogous pattern of expression. The results were presented as percentage of cells expressing RFP in FIGURE 3, (n = 5). Both oxysterol agonists tested, 22(R)-hydroxycholesterol and the synthetic T0 901 317 compound, were quite strong inducers of *COX-2* transgene. Their potency at the concentrations tested was comparable to IL-1β. However, expression of the transgene upon stimulation did not discriminate for the allelic variant tested.

DISCUSSION Distribution of the *COX-2* $_{-765}$G>C genotypes observed in control subjects is in line with the results of our previous study on the Polish population[12] and with other studies on the white European and American populations. In all of these studies, the minor C allele frequency ranged from 0.15 to 0.18.[10,11,14,15] A higher frequency of the variant allele was reported only in Italian[8] and Spanish[16] controls with the minor C allele frequency of 0.25. A higher frequency of C variant allele, ranging from 0.32 to 0.42 was observed in African Americans.[11,15]

It has been reported that $_{-765}$C allele might protect against myocardial infarction.[8] C allele was

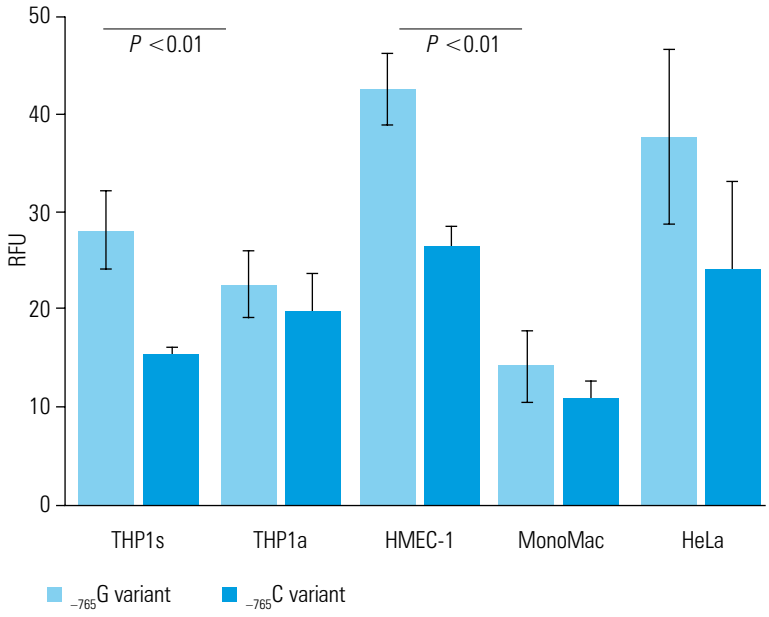

FIGURE 2 Differences in the expression of red fluorescence protein (RFP) transgene in several different transiently transfected cell lines. Cells were initially gated for cotransfected green fluorescence marker of the reaction efficiency. Abbreviations: RFU – relative fluorescence units of cells expressing RFP

also thought to be associated with lower levels of inflammatory markers such as CRP and IL-6 in cardiac, cerebrovascular, and hypercholesterolemic patients.[6,16] In contrast to these associations, we found no evidence for risk of prior myocardial infarction due to the presence of the variant *COX-2* allele. Hegener et al.,[10] Huuskonen et al.,[14] and Lee et al.[15] reached similar conclusions. Furthermore, Kushaka et al.[11] has recently reported that $_{-765}$C allele is in fact a risk factor for stroke in African Americans. Observations from Finland[14] also suggest that C allele is a risk factor for CAD. Thus, middle-aged Finnish men who died suddenly and carried the minor frequency C allele had larger areas of complicated coronary lesions and a higher number of coronary arteries with over 50% stenosis, compared with men with GG genotypes.

Our results indicate that the *COX-2* promoter polymorphism $_{-765}$G>C is associated with systemic production of PGE_2 in patients with stable coronary artery disease. The presence of C allele is associated with higher PGE_2 biosynthesis than the presence of G allele alone. We used the specific and highly sensitive method for measurement of stable urinary PGE_2 metabolite considered to reflect the systemic PGE_2 production. In the studied patients, as observed in healthy subjects, tetranor-PGE-M excretion was higher in men (9.19 ng/mg creatinine; 7.28–11.1, 95% CI) than in women (7.59 ng/mg creatinine; 3.94–11.24, 95% CI; $P = 0.1$). Urinary excretion of 6-keto-$PGF_{1\alpha}$, one of the inactivation products of PGI_2, was not influenced by the *COX-2* polymorphism. However, in contrast to tetranor-PGE-M, 6-keto-$PGF_{1\alpha}$ predominantly reflects renal biosynthesis of prostacyclin.[17]

We suggest that a large part of tetranor-PGE-M, which we studied, could be generated by stimulated monocytes/macrophages. Monocyte-derived macrophages are involved in all stages of atherosclerotic lesion development.[18,19] PGE_2 is a predominant prostanoid synthesized via monocytic COX-2. Its production by stimulated blood monocytes is associated with the onset and progression of carotid atherosclerosis. Exclusively monocytes/macrophages express on their surface CD163, a 130-kDa hemoglobin scavenger receptor. $CD163^+$ macrophages are present in atherosclerotic lesions and several lines of evidence link them to the development of atherosclerosis. sCD163 is a normal constituent of plasma and is generated by the proteolytic cleavage of CD163 at the cell surface. Its plasma levels increase significantly with CAD progression.[19] Importantly, sCD163 has been found to be a predictor of CAD extension, independently of the conventional risk factors.[19] In all our patients with CAD, this novel plasma marker of coronary atherosclerotic burden correlated significantly with tetranor-PGE-M. Moreover, sCD163 levels were greater in patients carrying $_{-765}$C allele by 60.5% compared with allele G homozygotes.

In vitro experiments, in which we tested the expression pattern of the transgene, engineered to produce red-fluorescence protein under

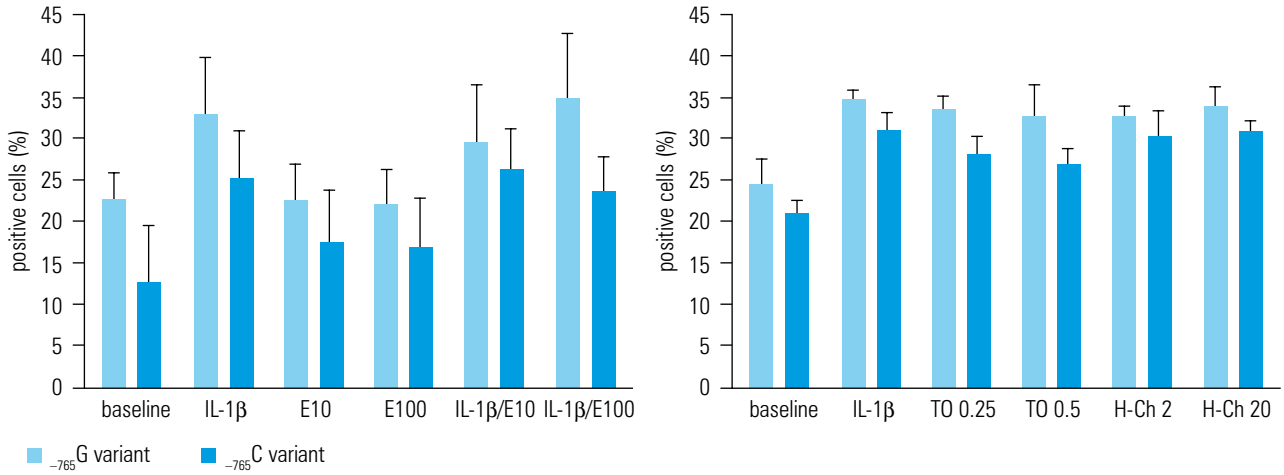

FIGURE 3 Induction of expression of red fluorescence protein transgene in stably transfected and differentiated THP-1 cell line. The stimuli included interleukin 1β (IL-1β; 10 ng/ml), estradiol (E 10 and 100 nM), 22(R)-hydroxycholesterol (H-Ch; 0.2 and 20 μM), and its analogue T0 901 317 (TO; 0.25 and 2.5 μM).

the control of either allelic variant of *COX-2* regulatory region, were generally in line with the original report by Papafili et al.[6] We confirmed higher COX-2 induction in HeLa cells stimulated with bacterial LPS, conditions under which $_{-765}$G allele showed increased expression. Using cell lines of endothelial origin (HMEC-1) and of monocyte/macrophage lineage (MonoMac and THP-1), we replicated this finding across a spectrum of biological compounds known to induce COX-2 expression. Despite numerous experiments, including oxysterols hinted by contribution of total serum cholesterol to the systemic PGE_2 biosynthesis, our cellular models seem far from a natural human setting, because most of COX-2 induction that we observed was still tenfold less, as compared to ex vivo experiments on blood-derived monocytes.[12] Thus, established cell lines are missing an important factor, which was responsible for our previous observation on a spectacular overproduction of PGE_2 in blood-derived monocytes in subjects carrying $_{-765}$C allele. In a recent report on COX-2 induction in adherent macrophages,[20] one of these humoral factors was identified as transforming growth factor-β, released by activated blood platelets. Increased activity of COX-2 in macrophages results in overproduction of prostanoids, which act not only on the systemic level but also in autocrine or paracrine manner. As the constructs we tested produced an inert marker protein instead COX-2, no prostanoid feedback was included in our model. Using peripheral blood monocytes, Skarke et al.[21] observed increased abundance of *COX-2* transcripts in $_{-765}$CC subjects following LPS stimulation, a pattern which required a prolonged cell activation.

In summary, we observed that CAD patients carrying a genetic variant of COX-2 $_{-765}$C produce greater amounts of PGE_2, and this overproduction is accompanied by enhanced serum levels of sCD163, a marker of macrophage/monophage activation. In vitro, the variant $_{-765}$C allele is less responsive to a wide range of cyclooxygenase inducers than $_{-765}$G allele, probably because this model lacks a pivotal factor that activates macrophages within the arterial wall.

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ARTYKUŁ ORYGINALNY

Polimorfizm regionu promotorowego genu dla cyklooksygenazy-2 wpływa na odpowiedź zapalną w stabilnej chorobie wieńcowej

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SŁOWA KLUCZOWE

choroba niedokrwienna serca, cyklooksygenaza-2, polimorfizm genetyczny, prostaglandyna E_2, zapalenie

STRESZCZENIE

WPROWADZENIE Mediatory prozapalne, w tym prostanoidy syntezowane przez indukowalną cyklooksygenazę-2 (COX-2), mają istotny udział w rozwoju miażdżycy. W regionie kontrolującym ekspresję genu COX-2 jest obecny częsty polimorfizm pojedynczego nukleotydu $_{-765}$G>C, którego znaczenie czynnościowe i wpływ na cechy fenotypu miażdżycy nie zostały do końca określone.

CELE Celem badania była ocena związku między polimorfizmem $_{-765}$G>C genu COX-2 a odpowiedzią zapalną u chorych na stabilną chorobę wieńcową.

PACJENCI I METODY U chorych na stabilną chorobę wieńcową zbadano ogólnoustrojową biosyntezę prostaglandyny E_2 (PGE_2), poziom w surowicy rozpuszczalnego białka CD163, będącego markerem aktywacji monocytów/makrofagów, oraz polimorfizm $_{-765}$G>C genu COX-2. Zbadano również czynnościowe efekty polimorfizmu regionu regulatorowego COX-2 z zastosowaniem linii komórkowych, w których dononano transfekcji konstruktów z genem białka czerwonej fluorescencji jako genem reporterowym pod kontrolą znacznego segmentu obszaru regulatorowego COX-2.

WYNIKI U chorych na stabilną chorobą wieńcową, w przypadku nosicielstwa wariantu $_{-765}$C obserwowano wzmożone wydalanie metabolitu PGE_2 z moczem oraz wyższy poziom rozpuszczalnego białka CD163 w surowicy, niż u chorych z allelem $_{-765}$G. W przeciwieństwie do obserwacji w modelu klinicznym, badania czynnościowe *in vitro* wykazały, że wariant $_{-765}$C jest mniej reaktywny niż wariant $_{-765}$G w odpowiedzi na stymulację szeregiem różnych czynników indukujących ekspresję COX-2.

WNIOSKI Aktywowane makrofagi/monocyty odgrywają znaczną rolę w ogólnoustrojowej biosyntezie całkowitej puli PGE_2 u chorych ze stabilną chorobą wieńcową. Dokładny mechanizm aktywacji tego szlaku w chorobie niedokrwiennej serca wymaga dalszych badań ze względu na rozbieżności pomiędzy wynikami badań klinicznych a wynikami badań czynnościowych *in vitro* nad poliformizmem $_{-765}$G>C genu COX-2.

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