

Association of *COX-2* gene haplotypes with prostaglandins production in bronchial asthma

To the Editor:

Common single nucleotide polymorphisms (SNPs) are linked within a gene locus and are inherited as several gene variants of different functional properties. The haplotype analysis emerged from genomic studies as an important clinical tool. In the lung, inflammation leads to induction of *COX-2* gene and to enhanced production of both proinflammatory and anti-inflammatory eicosanoids. Recently, we reported on a possible functional importance of the promoter polymorphism of *COX-2* G₋₇₆₅C in subjects with asthma.¹ Peripheral blood monocytes' capacity for biosynthesis of prostaglandin (PG) E₂ and PGD₂ was increased 10-fold in CC homozygotes compared with GG ones. Similar genotype frequencies of *COX-2* G₋₇₆₅C

TABLE I. Clinical characteristics of studied subjects with asthma*

Genotype of <i>COX-2</i> $-765G \rightarrow C$ (selector)	CC	CG	GG
Age (y)	33.6 ± 8.1	42.3 ± 8.7	47.6 ± 11.4
Sex (F/M ratio)	8/0	8/0	9/0
Duration of asthma (y)	10.5 ± 6.3	8.2 ± 5.7	6.3 ± 5.7
Atopy (yes/no)	7/1	5/3	6/3
Blood eosinophil count (per mm ³)	258.6 ± 216.3	223.2 ± 203.3	197 ± 111.2
Serum IgE (UI/mL)	407.7 ± 531.2	220.3 ± 238.2	138.25 ± 154.1
FEV ₁ (% of predicted value)	96.8 ± 14.4	90.1 ± 13.2	92.8 ± 15.4
Inhaled steroids (yes/no)	7/1	6/2	7/2
Dose of inhaled steroids (μg/d)	516.6 ± 466.3	331.2 ± 290.2	283.2 ± 208.6
Patients on oral steroids	2	2	2

*Means ± SDs.

TABLE II. *COX-2* haplotypes and prostaglandins concentrations in monocyte cultures§

Compound genotype	PGE ₂	PGD ₂	PGE ₂ -LPS	PGD ₂ -LPS
C-C/C-C (n = 7)	0.79 ± 0.19‡/‡	0.048 ± 0.03‡/NS	1.68 ± 0.25‡/†	0.113 ± 0.03‡/NS
C-C/other (n = 7)	0.24 ± 0.03 ^{NS}	0.024 ± 0.04*	1.05 ± 0.30†	0.073 ± 0.03†
Other/other (n = 11)	0.07 ± 0.03	0.05 ± 0.03	0.19 ± 0.09	0.013 ± 0.0

**P* < .05.†*P* < .01.‡*P* < .001.

§Means ± SDs in ng/mL. Other = G-T, G-C, or C-T Differences between C-C positives and C-C negative (other/other) genotype for prostaglandin concentrations (log-transformed data) were contrasted using the Tukey post hoc test. At C-C/C-C genotype, the first probability refers to the difference with the C-C negative one, the second to the C-C heterozygous one.

polymorphism were subsequently found in a large Australian study²; however, the authors did not investigate sex associations with asthma. Because *COX-2* gene has numerous single nucleotide polymorphisms, and our functional findings of G₋₇₆₅C were in disagreement with previous work by Papafili et al.³ we wondered whether particular haplotypes of *COX-2* could better correlate with prostaglandins' biosynthetic capacity in subjects with asthma. Out of several SNP candidates, a T → C transition within the 3'-untranslated region (COX2.8473) seemed particularly interesting. This SNP was found to be associated with increased risk for nonsmall cell lung cancer.⁴ As an explanatory mechanism, the authors proposed stabilization of the mRNA molecule due to disruption by T → C transition of the adenine-uracil-rich motif, conferring a signal for *COX-2* transcript degradation.

Subjects with asthma, all women, were studied for capacity of PGE₂ and PGD₂ biosynthesis in peripheral blood monocytes by using a haplotype-based approach. The methods were described previously.¹ In addition to $-765CC$ homozygotes (n = 8) and $-765GG$ homozygotes (n = 9), the peripheral blood monocyte cultures were completed in 8 women with asthma heterozygous for this locus (Table I). Genomic DNA was genotyped for $-765G \rightarrow C$ polymorphism as described previously¹ and for COX2.8473 SNP using a similar PCR-RFLP method. Briefly, a 177-bp PCR product was amplified with primers: 5'-GAAATTTTAAAGTACTTTTGAT and 5'-CTTTTACAGGTGATTCTACCC. A mutagenic primer (underlined nucleotide) introduced a restriction

site for *BclI* restriction nuclease; thus, C allele was cut to 156 and 21 bp. Combined genotypes were also ascertained in a sample of 76 individuals without asthma. Haplotype frequencies were calculated by a maximum likelihood method using a Markov chain of 10,000 iterations with 3 bootstrap replicates,⁵ and the number of copies of C-C haplotype was selected as an independent variable for ANOVA tests.

In subjects with asthma, the C-C haplotype (locus order G₋₇₆₅C – COX2.8473T → C) had the highest frequency, 41.7%, followed by G-T (33.7%), G-C (18.3%), and C-T (6.3%) because of preselection of $-765CC$ homozygous subjects. However, when corrected for this selection bias, haplotypes did not differ from the ones in the population sample (C-C, 12.0% vs 12.3%; G-T, 55.9% vs 64.9%; G-C, 30.3% vs 21.3%; and C-T, 1.8% vs 1.5%). The 2 *COX-2* loci were in linkage disequilibrium (*P* < .0001) in controls, suggesting a tight linkage of COX2.8473 T → C SNP with $-765C$ allele. Linkage disequilibrium between the loci was also significant in the subjects with asthma (*P* = .0005). Among subjects with asthma, 7 individuals had 2 copies of the C-C haplotype, 7 had 1 copy of C-C, and 11 had other haplotypes.

The mean concentrations of PGs in monocyte cultures and post hoc comparisons of ANOVA for diplotype classes are presented in Table II. ANOVA on log-transformed PG data was highly significant (*P* < .001) for the number of C-C haplotype copies as an independent variable. Comparison of PG levels in cases with the most contrasting wild-type G-T/G-T (n = 4) and variant

C-C/C-C genotypes ($n = 7$) revealed a 19.8-fold increase of PGE_2 ($P < .001$) and a 7.4-fold increase of PGD_2 ($P = .024$) in unstimulated monocytes with the variant genotype. In LPS-stimulated cells, these differences were respectively 19.6-fold and 11.5-fold ($P = .001$).

There was uniformly strong linear correlation between the number of C-C haplotype copies and capacity for biosynthesis of PGE_2 (log-transformed data, Pearson $r = 0.80$; $P < .001$) and PGD_2 ($r = 0.69$; $P = .002$). Stimulation of monocytes with LPS resulted in a very similar correlation for PGE_2 ($r = 0.80$; $P < .001$) and PGD_2 ($r = 0.78$; $P < .001$). The C-C haplotype had no effect on magnitude of PG induction by LPS.

We analyzed haplotypes of 2 functional SNP candidates within *COX-2* gene. One of the loci— $G_{-765}C$ —was previously studied by us in opposite homozygotes with asthma. We now expanded this group, adding 8 subjects heterozygous for this SNP. In these heterozygotes, PG biosynthetic capacity of monocytes was in between CC and GG homozygotes (data not shown). Haplotypes of the 2 SNPs were reconstructed, and PG levels in monocyte cultures were reanalyzed using arbitrary classes of compound genotype. The haplotype variant C-C was expected^{1,4} to associate with the highest activity of *COX-2*. Indeed, there was a very strong linear correlation between the number of C-C haplotype copies and PG levels in monocyte cultures. Because our subjects with asthma were selected for functional studies of *COX-2* polymorphisms by $G_{-765}C$ genotypes, estimated haplotype frequencies are not representative for a random sample of subjects. The haplotypes of *COX-2* were in linkage disequilibrium both in a group of controls without asthma and in subjects with asthma. The difference in PG production by peripheral blood monocytes of subjects with asthma with extreme compound genotypes G-T/G-T and C-C/C-C was striking. Our data suggest an additive effect of C-C *COX-2* haplotype on gain of function, because the difference between the extreme compound genotypes (C-C vs G-T) in PGE_2 levels was by 65% greater than the one between opposite homozygotes $-765CC$ and $-765GG$.¹ The presented haplotype approach was validated by several pharmacogenomic studies⁶ and identified the *COX-2* variant related to overproduction of prostaglandins. No complete study on the interaction between the 3' untranslated region $\text{COX2.8473T} \rightarrow \text{C}$ polymorphism and promoter $G_{-675}C$ variants was possible because of a low frequency of some haplotypes. However, altered promoter activity of *COX-2* gene related to $G_{-765}C$ SNP seemed to be predominant but additive with stabilization of the mRNA transcript as a result of $\text{COX2.8473 T} \rightarrow \text{C}$. Because *COX-2* activity plays a pivotal role in inflammation, this overproducing genetic C-C variant of the gene seems worthy of study in several human pathologies that, apart from asthma, are mediated by the inducible activity of the isoenzyme.

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REFERENCES

1. Szczeklik W, Sanak M, Szczeklik A. Functional effects and gender association of *COX-2* polymorphism $G_{-765}C$ in bronchial asthma. *J Allergy Clin Immunol* 2004;114:248-53.
2. Shi J, Misso NL, Duffy DL, Thompson PJ, Kedda M-A. A functional polymorphism in the promoter region of the cyclooxygenase-2 gene is not associated with asthma and atopy in an Australian population. *Clin Exp Allergy* 2004;34:1714-8.
3. Papafili A, Hill MR, Brull DJ, McNulty RJ, Marshall RP, Humphries SE, et al. Common promoter variant in cyclooxygenase-2 represses gene expression: evidence of role in acute-phase inflammatory response. *Arterioscler Thromb Vasc Biol* 2002;22:1631-6.
4. Campa D, Zienolddiny S, Maggini V, Skaug V, Haugen A, Canzian F. Association of a common polymorphism in the cyclooxygenase-2 gene with risk of non-small cell lung cancer. *Carcinogenesis* 2004;25:229-35.
5. Schneider S, Roessli D, Excoffier L. Arlequin Ver. 2.00: A Software for Population Genetics Data Analysis. 2002. Available at: <http://anthropologie.unige.ch/arlequin/>. Accessed December 15, 2004.
6. Ahmadi KR, Weale ME, Xue ZY, Soranzo N, Yarnall DP, Briley JD, et al. A single nucleotide polymorphism tagging set for human drug metabolism and transport. *Nat Genet* 2005;37:84-9.

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