a

b

c

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* $G_{-765}C$ in subjects with asthma.[1] Peripheral blood monocytes' capacity for biosynthesis of prostaglandin (PG) E_2 and PGD_2 was increased 10-fold in CC homozygotes compared with GG ones. Similar genotype frequencies of *COX-2* $G_{-765}C$

J ALLERGY CLIN IMMUNOL
JULY 2005

TABLE I. Clinical characteristics of studied subjects with asthma*

Genotype of *COX-2* $_{-765}$G→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 study[2]; however, the authors did not investigate sex associations with asthma. Because *COX-2* gene has numerous single nucleotide polymorphisms, and our functional findings of $G_{-765}C$ 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 PGE_2 and PGD_2 biosynthesis in peripheral blood monocytes by using a haplotype-based approach. The methods were described previously.[1] In addition to $_{-765}$CC homozygotes (n = 8) and $_{-765}$GG 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 $_{-765}$G→C polymorphism as described previously[1] 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 $G_{-765}C$ – 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 $_{-765}$CC 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 $_{-765}$C 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 $_{-765}$CC and $_{-765}$GG.[1] The presented haplotype approach was validated by several pharmacogenomic studies[6] and identified the *COX-2* variant related to overproduction of prostaglandins. No complete study on the interaction between the 3′ untranslated region COX2.8473T→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 COX2.8473 T→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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The work benefited from the experience gained in the European Community Program Global Allergy and Asthma European Network. Supported by Jagiellonian University Grants PKL/8 and KL/611 and Polpharma Foundation Grant F/P/4.

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Available online April 25, 2005.
doi:10.1016/j.jaci.2005.03.010

Childhood cat exposure–related tolerance is associated with *IL1A* and *IL10* polymorphisms

To the Editor:

Atopic parents have been advised not to acquire pets to reduce their child's risk of allergic diseases. However, it has been shown that the presence of a cat in the home might decrease the risk of sensitization to cat allergen,[1] and exposure to domestic pets in the first year of life significantly reduces the risk of allergic sensitization in children.[2,3]

IL-1 is a potent inducer of IL-10 and also plays a role in early T-cell priming, which has been considered to be one key point of interest in the development of tolerance.[4] We have previously studied *IL1A* (G/T base exchange at +4845) and *IL10* promoter region polymorphism in atopy and asthma.[5,6]

Now we investigated whether the genes encoding the cytokines IL-1α *(IL1A)* and IL-10 *(IL10)* affect exposure-related tolerance. Therefore we analyzed the association of cat and dog exposure in childhood with the sensitization to cat and dog allergens in a population-based sample of adult asthmatic subjects (n = 245) and their nonasthmatic control subjects (n = 405). Detailed information on the study population has been presented previously in this journal.[5]