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ORIGINAL PAPER

Cyclooxygenase-2 gene polymorphisms in an Australian population: association of the −1195G > A promoter polymorphism with mild asthma

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Summary

Background Cyclooxygenase (COX)-2 is an inducible enzyme responsible for catalysing the formation of prostaglandins (PGs) in settings of inflammation. Single nucleotide polymorphisms (SNPs) of the COX-2 gene may influence gene transcription and PG production in the asthmatic airway.

Objective To evaluate the frequencies of COX-2 SNPs in an Australian Caucasian population, and determine potential associations between common COX-2 promoter SNPs and asthma, asthma severity and aspirin-intolerant asthma (AIA).

Methods The frequencies of 25 COX-2 SNPs were determined in a random population ($n = 176$). The SNPs with a minor allele frequency of >10% were then studied in asthmatic ($n = 663$), non-asthmatic controls ($n = 513$) and AIA subjects ($n = 58$). Genotype, allele and haplotype associations were assessed. Functional assessment of SNPs was performed by transfection into HeLa cells measured using the luciferase dual-reporter assay system.

Results Eighteen COX-2 SNPs were not detected, five were rare and two promoter SNPs, −1195G > A (rs689465), and −1290A > G (rs689466), were further studied. The A allele of the −1195 SNP was present at a significantly higher frequency among all asthmatic subjects ($P = 0.012$). Over 60% of the asthmatic individuals were −1195A homozygotes compared with 54.6% of the control subjects (odds ratio, 1.35; 95% CI, 1.06–1.72, $P = 0.03$). After classifying for severity, the mild asthmatics represented 64.6% of −1195AA individuals, the highest of all the asthma groups compared with 54.6% of the control subjects (odds ratio, 1.5; 95% CI, 1.12–2.02, $P = 0.02$). The −1290A/−1195G/−765G haplotype was associated with a reduced incidence of asthma (odds ratio, 0.76; 95% CI, 0.61–0.95, $P = 0.017$).

Conclusion The −1195G > A polymorphism appears to be associated with asthma, and in particular with mild asthma.

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Introduction

Asthma is a complex multifactorial respiratory disorder, characterized by sporadic and reversible bronchoconstriction, airway hyper-responsiveness, inflammation and/or excessive sensitivity of the airways to allergens and other trigger factors [1]. A complex interaction between genetic and environmental components leads to the expression of a wide spectrum of clinical asthma phenotypes [2–4]. Aspirin-intolerant asthma (AIA) is a subtype of asthma characterized by bronchoconstriction, chronic rhinosinusitis and nasal polyposis that occurs following the ingestion of aspirin and other non-steroidal anti-inflammatory drugs whose mode of action is through the inhibition of cyclooxygenase (COX) [5]. Several polymorphic loci in genes related to inflammation and immune responses

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have been postulated to play important roles in the pathogenesis of asthma [2] and as such may influence response to therapies [6].

Prostaglandins (PGs) are important mediators of airway inflammation in asthma [7]. The COX gene codes for the key enzyme that regulates PG synthesis by converting arachidonic acid to PGH_2, which is further metabolized to various prostanoids, including PGE_2 and PGD_2, depending on the presence of specific synthases in individual tissues [8, 9].

Of the three COX isoforms reported (COX-1, COX-2 and COX-3), COX-1 is considered to be a constitutive enzyme, responsible for generating PGs for cell–cell signalling, blood clotting, renal function, gastrointestinal integrity and tissue homeostasis [10, 11]. However, COX-1 may also be regulated [12, 13] and may play a role in pathophysiological processes in entities such as arthritis and cancer [10]. Previous work from this laboratory demonstrated that the c.22C > T coding region polymorphism in the COX-1 gene was weakly associated with atopy, but not with asthma [14].

Conversely, the COX-2 isoform is an inducible enzyme responsible for the synthesis of PGs in a variety of inflammatory states. COX-2 is expressed in human airway epithelium and its expression varies under different physiological and pathological conditions [15–17]. In asthmatic patients, COX-2 expression is increased in induced sputum cells and in the bronchial epithelium [18, 19]. Based on these findings and other data, it was hypothesized that single nucleotide polymorphisms (SNPs) in the COX-2 gene may modify COX-2 transcription and expression, leading to a worsening or an improved asthma phenotype and increased susceptibility to AIA.

Previous work has shown that the functional −765G > C polymorphism in the COX-2 promoter is not associated with asthma or atopy in an Australian population [20]. However, in an asthmatic population from Krakow, Poland, −765CC homozygosity was associated with female gender and with a 10-fold greater production of PGE_2 and PGD_2 by monocytes [21]. Other reports have shown that the −1195G > A promoter SNP also alters COX-2 transcription and was associated with oesophageal cancer in a Han Chinese population [22]. It would not be unreasonable to suggest that an imbalance in these inflammatory mediators could result in a predisposition to asthma; however, the potential association of these SNPs or that of the −765G > C/−1195G > A haplotype with asthma has not been assessed previously in an Australian Caucasian population.

The aims of this study were to determine the frequencies of the COX-2 promoter and coding region polymorphisms in an Australian Caucasian population and to investigate the potential associations between relatively frequent SNPs and haplotypes with asthma, asthma severity and AIA by genotyping a large well-characterized population of asthmatic and non-asthmatic subjects.

Materials and methods

Subjects

To determine the frequencies of COX-2 polymorphisms in the general population, genomic DNA was isolated from peripheral blood of a random Australian Caucasian population of blood donors ($n = 176$) at the Red Cross Blood Bank, Perth, WA. In addition, 513 healthy control individuals with no history of asthma and 663 patients with asthma, comprising 322 subjects with mild, 253 with moderate and 88 with severe asthma, as well as 58 individuals with AIA participated in the association studies. Asthma was defined as respiratory physician-diagnosed asthma. All subjects were unrelated, and were between 20 and 89 years of age. The inclusion and exclusion criteria for recruitment of participants for the association study have been described in detail previously [23]. Blood samples (10 mL) were collected from each of the participants for DNA isolation and genotyping. The study protocol was approved by the Human Research Ethics Committees at Sir Charles Gairdner Hospital, Perth, The Prince Charles Hospital, Brisbane, and the Monash Medical Centre, Melbourne.

Classification of clinical phenotypes

Patients were classified as having mild, moderate or severe asthma, according to the criteria specified by the Australian National Asthma Council and the US National Institutes of Health [24, 25], as described in detail previously [20, 23]. Asthmatic patients were also classified as aspirin tolerant or AIA using a validated questionnaire that has been used by us previously to assess the prevalence of aspirin intolerance in asthmatic patients [26].

Selection of polymorphisms

This study focused on polymorphisms located in the promoter, 5′-untranslated and coding regions of the COX-2 gene (GenBank accession no. D28235). A total of 25 SNPs were identified by searching the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov/SNP) and SNPper databases [27]. The locations of SNPs in the gene were confirmed using the basic local alignment search tool program to align the dbSNP cluster sequences with the COX-2 DNA sequence [28]. For coding region SNPs, the positions in the cDNA sequence and the corresponding amino acid residues in the protein were confirmed using the translated coding sequence (GenBank accession no. NM_000963). Polymorphisms chosen for further study were those for which the frequencies of the minor alleles were > 10% in the random Caucasian subjects.

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Genotype analysis

Genomic DNA was extracted from a buffy coat using a DNA extraction kit (Qiagen, Melbourne, Australia), according to the manufacturer's instructions. Genotyping was performed by PCR and restriction fragment length polymorphism analysis. The proximal promoter region, including the −1195G > A and −1290A > G variants, was amplified using the primers: sense; −5′-GGC TTC CTA GAT GAG ATG GA-3′ and antisense, −5′-GTG ACC ATG GAT CAA AGT AC-3′. PCR was performed in a 20 μL reaction containing 40 ng DNA, 1 × PCR buffer (Qiagen), 2.5 mM $MgCl_2$, 400 μM of each dNTP (Promega Madison, WI, USA), 20 pmol of each primer and 0.4 U of Taq DNA polymerase (Qiagen). The optimized PCR conditions to denature were at 94 °C for 5 min, followed by 20 cycles at 94 °C for 30 s, 65 °C for 30 s (decreasing by 0.3 °C per cycle) and 72 °C for 30 s. This was followed by 15 cycles at 94 °C for 30 s, 55 °C for 30 s and 72 °C for 30 s. The final extension step was continued for another 5 min at 72 °C.

The −1195G > A variant was genotyped by incubation of 10 μL PCR product with *Pvu*II restriction enzyme (5 U, New England Biolabs, Beverly, MA, USA) in 1 × buffer (total volume 20 μL) at 37 °C for 4 h. The −1290A > G variant was identified using *BsrG*I (5 U, New England Biolabs) in 1 × buffer (total volume 20 μL) at 60 °C for 4 h. Restriction products were separated in 2.5% agarose gels. Repeat digestions were performed when there was any doubt regarding the visualization of all fragments, to ensure that these represented true heterozygotes rather than random partial digests. PCR products were sequenced to confirm the SNPs at −1195G > A and −1290A > G.

Construction of reporter plasmids and functional assays

To test the functional activity of the −1195G > A polymorphism, portions of the COX-2 promoter encompassing base pairs −1667 to +10 and including the transcription start site and either with haplotype loci −1290A/−1195G/−765G or −1290A/−1195A/−765G were amplified from genomic DNA. The primers used for generating these fragments were sense: 5′-GCGCGCACGCGTGATCCACGCTCTTAGTTGAA-3′ and antisense: 5′-GCGCGCAAGCTTACAATTGGTCGCTAACCGAG-3′, which contain 5′-*Mlu*I and 3′-*Hind*III restriction sites (underlined sequences), respectively. To ensure high-fidelity amplification, the Elongase Enzyme Mix (1 μL, Invitrogen, Melbourne, Australia) was used in the PCR, which was performed in 50 μL reactions containing 100 ng DNA, 0.5 × PCR buffers A and B (Invitrogen), 400 μM of each dNTP (Promega) and 10 pmol of each primer. PCR conditions included an initial denaturation at 94 °C for 30 s and 30 cycles at 94 °C for 30 s, 61 °C for 30 s and 68 °C for 100 s. PCR products were double digested using *Mlu*I and *Hind*III, and then ligated into a linearized pGL3-Basic vector (Promega) containing a Firefly luciferase reporter gene. The inserts of the constructs were verified by sequencing before transient transfection.

Transfection was carried out using HeLa cells plus Lipofectamine reagent (Invitrogen) in F12K medium. Cells were co-transfected with 0.5 μg of reporter plasmid and 50 ng of pRL-CMV control vector (Promega) containing Renilla luciferase, which was used to correct for transfection efficiency. Transfection cells were harvested after 24 h and cell lysates were assayed sequentially for Firefly and Renilla luciferase activity using a dual-reporter assay system (Promega). Transfections were performed in quadruplicate for each plasmid construct. Changes in gene transcription were calculated relative to the corrected luciferase activity of the empty pGL3-Basic vector (control).

Transcription factor binding site prediction

The transcription factor binding site TRANSFAC [29] database was used to predict whether polymorphisms in the promoter region of the COX-2 gene might result in the gain or loss of putative transcription factor binding sites.

Statistical methods

Genotype and allele frequencies were calculated for each group of patients. Univariate comparisons of allele and genotype distributions were performed using χ^2 tests and logistic regression analysis with the R Statistics Program [30]. Hardy–Weinberg equilibrium analysis for each group was performed using the exact test, implemented in the R Statistics program. The haplotype analyses were performed using the haplo.stats package of Schaid et al. [31].

Results

Identification of single nucleotide polymorphisms in the cyclooxygenase-2gene

Twenty-five SNPs, 16 in the promoter, three in the 5′-UTR and six non-synonymous mutations in the coding region of the gene, were investigated. The reference SNP ID numbers, resulting amino acid substitutions and allele frequencies for these SNPs in the random Australian Caucasian population are presented in Table 1. The locations of the eight SNPs occurred in our population, including the one in the previous study, in the gene and the putative transcription factor binding sites affected, as predicted by TRANSFAC [29] are shown in Fig. 1. For 18 SNPs, the minor alleles were not detected, and for five SNPs the minor allele frequencies were < 3%. These relatively rare SNPs were not studied further. The remaining two promoter polymorphisms (−1195G > A and −1290A > G), located 1195 and 1290 bases upstream of the transcription start site, respectively, were further investigated in the asthmatic and control populations.

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Table 1. The polymorphisms in the promoter, coding region and 5′-UTR of the COX-2 gene and their allele frequencies in a random Australian Caucasian population ($n = 176$)

Ref SNP ID	SNP position*	Amino acid substitution†	Polymorphic allele frequency
Promoter			
rs4648251	−1612T > G	–	T = 1
rs2223626	−1588C > T	–	C = 0.997, T = 0.003
rs689462	−1527A > C	–	A = 0.97, C = 0.03
rs689465	−1290A > G	–	A = 0.87, G = 0.13
rs689466	−1195G > A	–	A = 0.79, G = 0.21
rs2745558	−1324C > T	–	C = 1
rs3918304	−1151A > G	–	A = 1
rs20415	−1132G > A	–	G = 1
rs20416	−985C > T	–	C = 1
rs4648254	−960A > G	–	A = 1
rs4648256	−714T > C	–	T = 1
rs20420	−512C > T	–	C = 1
rs20422	−407C > T	–	C = 1
rs20423	−368T > C	–	T = 1
rs5270	−163C > G	–	C = 0.997, G = 0.003
rs20424	−62C > G	–	C = 0.98, G = 0.02
Coding region			
rs20426	Exon 1 G > A	M1I	G = 1
rs3218622	Exon 6 G > A	R228H	G = 1
rs4648279	Exon 9 C > G	P428A	C = 1
rs5272	Exon 10 A > G	E488G	A = 1
rs5273	Exon 10 T > C	V511A	T = 1
rs2318625	Exon 10 G > A	G587A	G = 1
5′-UTR			
rs5271	+10T > G	–	T = 0.997, G = 0.003
rs4648279	+17T > A	–	T = 1
rs11567819	+72C > T	–	C = 1

*From transcription start site.

†From cDNA; promoter GenBank accession no. D28235; coding region GenBank accession no. NM_000963; 5′-UTR GenBank accession no. U04636.

COX, cyclooxygenase; SNP, single nucleotide polymorphisms.

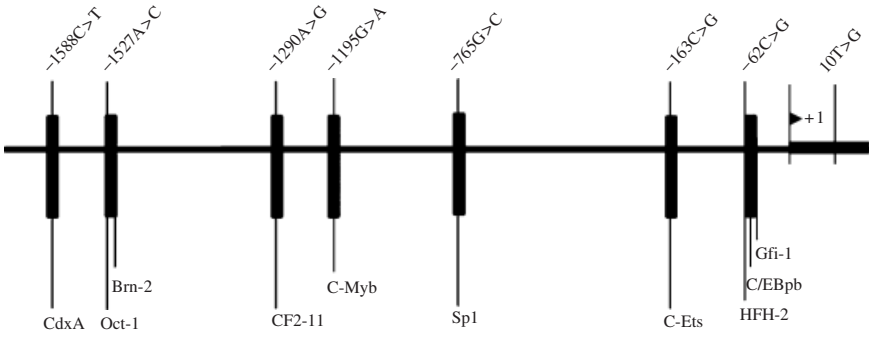


Fig. 1. Schematic representation of the promoter and 5′-UTR of the COX-2 gene showing the positions of the single nucleotide polymorphisms (SNPs) identified in the random Caucasian population and the putative transcription factor binding sites associated with these SNPs. The arrow and +1 denote the transcription start site.

Association analysis

The demographic and phenotypic characteristics of the asthmatic and control populations have been described in detail previously [20]. Genotype distributions and allele frequencies for the −1195G > A and −1290A > G polymorphisms in all asthmatic and control subjects are presented in Table 2. There was a significantly different distribution of genotypes at the −1195G > A polymorphism ($P = 0.03$), whereas the −1290A > G SNP was not associated with asthma and disease severity. The A

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Table 2. Genotype and allele frequencies for the two common COX-2 promoter polymorphisms in asthmatic and control subjects ($n = 1,176$)

Genotype or allele	Control Subjects ($n = 513$)	Asthmatic patients					
		All ($n = 663$)	Mild ($n = 322$)	Moderate ($n = 253$)	Severe ($n = 88$)	AIA ($n = 60$)	ATA ($n = 94$)
−1195G > A							
AA	54.6	62.2*	64.6†	60.5	58.0	60.0	61.7
AG	40.9	34.5	32.3	37.5	34.2	33.3	34.0
GG	4.5	3.3	3.1	2.0	8.0	6.7	4.3
A	75.0	79.4	80.1	79.7	75.0	76.7	78.7
G	25.0	20.5	19.3	20.3	25.0	23.3	21.3
−1290A > G							
AA	76.8	75.7	75.2	75.1	79.8	76.7	80.9
AG	20.9	23.1	23.6	23.3	20.5	23.3	18.1
GG	2.34	1.21	1.2	1.6	–	–	1.0
A	87.2	87.3	87.0	86.8	89.8	88.3	89.9
G	12.8	12.7	13.0	13.2	10.2	11.7	1.1

*$P = 0.03$ for genotype association comparing asthmatic with control subjects, and †$P = 0.02$ for genotype association comparing mild asthmatic with control subjects.

AIA, aspirin intolerant asthma; ATA, aspirin tolerant asthma; COX, cyclooxygenase.

allele of the −1195 SNP was present at a significantly higher frequency among all asthmatic subjects ($P = 0.012$). Furthermore, −1195AA homozygous individuals were significantly overrepresented among asthmatic patients (62.2%) compared with control subjects (54.6%) (OR 1.35, 95% CI 1.06–1.72, $P = 0.03$), particularly among the mild asthma phenotype cohort (64.6%, OR 1.5, 95% CI 1.12–2.02, $P = 0.02$). There was no gender interaction for the association between the −1195G > A SNP and asthma, nor was there any association with AIA. There were no allelic or genotype associations between the −1290A > G SNP and asthma, asthma severity or AIA.

Transcriptional activity of −1195GG and −1195AA constructs

When HeLa cells were transfected with the −1667 to +10 region of the COX-2 promoter, the luciferase activity generated by the −1195GG construct (0.043 ± 0.004 relative to control, $n = 4$) was 1.2-fold greater than that generated by the −1195AA construct (0.035 ± 0.001); however, this difference was not statistically significant.

Haplotype associations

The three common SNPs in the COX-2 promoter (−1290A > G/−1195G > A/−765G > C) have been shown previously to be in linkage disequilibrium [22], and haplotype associations for these three SNPs are shown in Table 3. The −1290A/−1195A/−765G haplotype was the most common and was present in 59.4% of the control subjects and 62.8% of the asthma patients. The −1290A/−1195G/−765G haplotype occurred in 23.1% of the control subjects but in only 19.5% of the asthmatic patients and this haplotype was associated with a reduced risk of asthma (OR 0.76, 95% CI 0.61–0.95, $P = 0.017$). None of the haplotypes showed a significant association with AIA.

Discussion

COX-2 is an important enzyme regulating the production of prostanoids that have both pro- and anti-inflammatory roles in normal physiology and in many disease states. Polymorphisms in the COX-2 gene and their potential association with various inflammatory diseases have therefore been the subject of many recent studies investigating the regulation of this gene [20–22]. In the current study, the frequencies of 25 SNPs reported previously in the LocusLink and SNPper databases were determined in a randomly selected population of 176 healthy Australian Caucasian subjects. Eighteen of these previously reported SNPs, including 10 in the promoter region six in the coding region and two in the 5′-UTR region, were not detected in this population.

The G alleles of the −1290A > G and −1195G > A promoter polymorphisms occurred with frequencies of 0.13 and 0.21, respectively, in the Australian Caucasian population. In contrast, among Han Chinese subjects, the frequency of the −1290G allele was 0.04 and that of the −1195G allele was 0.49 [22]. These results indicate a considerable variation between ethnic groups in the frequencies of polymorphisms in the COX-2 gene.

In a previous study by our group, the −765G > C SNP in the COX-2 promoter occurred with an allele frequency of ∼0.17 but was not associated with asthma in an Australian population [20]. In the present study, two additional promoter SNPs, −1290A > G and −1195G > A, were investigated for potential associations with

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Table 3. COX-2 promoter haplotype frequencies among asthmatic and non-asthmatic subjects and their association with asthma

Overall haplotype association with asthma −1290A > G / −1195G > A / −765G > C				*P*-value 0.162
	Frequency			
Haplotype	Non-asthmatic	Asthmatic	OR (95% CI)	*P*-value
AAG	0.5935	0.6283	1	
AGG	0.2313	0.1949	0.764 (0.613–0.952)	0.017
GAC	0.1134	0.1133	0.935 (0.714–1.226)	0.627
AAC	0.0317	0.0383	1.064 (0.658–1.722)	0.800
Rare	0.0301	0.0252	0.837 (0.484–1.447)	0.524

COX, cyclooxygenase.

asthma. The A allele or the wild-type allele of the −1195 SNP was present at a significantly higher frequency among all asthmatic subjects ($P = 0.012$). The A/A homozygous genotype was more frequent among asthmatic rather than non-asthmatic subjects. Furthermore, when asthmatic subjects were stratified according to disease severity, the A/A homozygous genotype was greatest in those with mild asthma rather than in other asthma severities. These results suggest that the presence of the −1195A allele is associated with an increased incidence of asthma, and more particularly, with a mild phenotype of asthma.

To explore the possible functional effect of the −1195G > A polymorphism, we conducted transfection studies using HeLa cells. These assays indicate that the −1195A construct only minimally alters the transcriptional activity of the COX-2 gene. This contrasts with the results of Zhang et al. [22] who showed that the presence of the wild-type −1195A allele increased COX-2 transcription in comparison with reporter plasmids containing the −1195G allele. The discrepancy between these studies is likely to be due to the use of different promoter constructs. In the present study, cells were transfected with constructs encompassing base pairs −1667 to +10, which includes the promoter region and a small portion of the 5′-UTR. In contrast, Zhang et al. [22] transfected HeLa cells with reporter constructs 467 bp longer encompassing not only a larger region of the promoter but also the entire 5′-UTR, plus exon 1 and part of intron 1 of the gene. It is feasible that the extra sequence in the Zhang construct included additional control elements such as modifiers or enhancers not present in our minimal promoter construct, thus explaining the differences between the two functional studies.

A number of binding sites for the c-Myb transcription factor are present in the COX-2 promoter region [32], and recent investigations have demonstrated that the −1195G > A SNP is located in a core recognition sequence for c-Myb [22]. C-Myb is known to have an important role in the haematopoietic and gastrointestinal systems, in which it regulates interactions with other proteins as well as the expression of a variety of genes involved in cell division, differentiation and survival [33]. The presence of the −1195A wild-type allele but not the −1195G minor allele results in binding of c-Myb to the COX-2 promoter; therefore, it might be expected that transcription of COX-2 will be increased in individuals who are homozygous or heterozygous for the −1195A allele [22]. The finding in the present study that individuals carrying the −1195A allele were more likely to have asthma suggests an association between increased transcription of COX-2 and the incidence of asthma. This finding is supported by previous observations demonstrating that COX-2 expression is increased in induced sputum, macrophages, dendritic cells and airway epithelium of asthmatic patients compared with non-asthmatic subjects [13, 18, 19].

While the −1195G > A polymorphism may be one of the factors contributing to increased COX-2 expression in asthma, the consequences of increased COX-2 enzyme activity are likely to vary in different tissues and cells, depending on whether the major end-product of COX-2 activity is PGE_2 or PGD_2 or other prostanoids. PGE_2 has a predominantly anti-inflammatory role in the airways [9], and protects against allergen-induced bronchoconstriction and inflammation [34, 35], but also enhances the survival of eosinophils [19, 36]. Although PGD_2 causes bronchoconstriction and has generally been considered to be a pro-inflammatory mediator [37], more recent evidence suggests a potentially important anti-inflammatory role for PGD_2 and its metabolites, particularly in relation to eosinophil function [38]. Accordingly, the biological consequences of increased COX-2 expression in asthmatic patients, whether resulting from the −1195G > A polymorphism, some other mechanism or both, are likely to be complex. Moreover, this may also explain the association of −1195AA homozygosity with mild rather than moderate or severe asthma, as increased capacity to produce beneficial anti-inflammatory PGE_2 through up-regulation of COX-2 activity may contribute to a more mild asthma phenotype.

In the present study, haplotype analysis showed that the presence of the −1290A and −1195G alleles in combination with the −765G allele was associated with

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a decreased risk of asthma, supporting the concept that the presence of both −1195G and −765G alleles in the promoter may be associated with altered COX-2 transcription and PG synthesis.

Previous studies have suggested that transcriptional regulation of COX-2 and a deficiency in COX-2-mediated PGE_2 production may contribute to the pathogenesis of AIA [39, 40]. However, this study did not show associations between the −1195G > A and −1290A > G promoter polymorphisms and AIA, and previous studies also did not show an association between AIA and the −765G > C polymorphism [20, 21], although −765CC homozygosity among AIA patients was associated with more severe disease [21]. The lack of a haplotypic association in the current study between AIA and these three promoter SNPs suggests that they are not important determinants of COX-2 function in the context of its putative role in the pathogenesis of AIA.

This is the first study to investigate comprehensively the SNPs in the COX-2 gene and their potential associations with asthma, asthma severity and AIA. Only seven of the 25 SNPs investigated were present in a random Australian Caucasian population. Subjects with the −1195AA allele in the COX-2 promoter showed an increased incidence of asthma, and the association with mild asthma was particularly strong. In addition, subjects with the −1290A/−1195G/−765G haplotype showed a reduced incidence of asthma, suggesting that these COX-2 promoter polymorphisms may contribute to altered expression of the enzyme activity with potential pro- and anti-inflammatory consequences in the airways of asthmatic patients. Further studies, including measurements of PG production from cells of asthmatic patients who are homozygous for the −1195G > A SNP is required in order to confirm the functional significance of the association of this COX-2 promoter polymorphism with asthma.

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