Allergy 2006: 61: 543–548

© 2006 The Authors
Journal compliation © 2006 Blackwell Munksgaard

DOI: 10.1111/j.1398-9995.2006.01025.x

Original article

Promoter genetic variants of prostanoid DP receptor (*PTGDR*) gene in patients with asthma

Background: *PTGDR* gene has been identified as an asthma-susceptibility gene. Recently, functional genetic variants have been associated with asthma. The objective of this work was to study −549T > C, −441C > T and −197T > C *PTGDR* promoter polymorphisms in a Spanish population.
Methods: In this study, 197 Caucasian individuals were included. Asthma was specialist-physician diagnosed according to the American Thoracic Society (ATS) criteria and classified following the Global Initiative for Asthma (GINA) guidelines. Skin prick tests were performed in all patients. The polymorphisms were analyzed by direct sequencing.
Results: −197T > C polymorphism was significantly associated with asthma [Fisher's *P*-value = 0.007, Monte Carlo *P*-value (10^4 simulations) = 0.004]. Multivariate analysis adjusted for age and sex confirmed this association with an increased risk of asthma (OR, 3.06; 95% CI, 1.28–7.32; *P*-value = 0.012). CCT CCC diplotype was associated with asthma (*P*-value < 0.0001; OR, 1.15; 95% CI, 1.07–1.23), specifically with allergic asthma (*P*-value < 0.0001). CCT CCC diplotype is unambiguous. All individuals carrying this diplotype had asthma.
Conclusion: We identified a specific promoter variant of *PTGDR* that could be associated with asthma. This diplotype is a combination of the two highest transcriptional efficiency haplotypes, recently described. Our *in vivo* results would support for the first time what was demonstrated *in vitro* about high-transcriptional efficiency *PTGDR* haplotypes in asthma.

C. Sanz[1], M. Isidoro-García[2], I. Dávila[1], E. Moreno[1], E. Laffond[1], C. Ávila[3], F. Lorente[1]

[1]Department of Allergy, University Hospital of Salamanca; [2]Molecular Medicine Unit, Department of Medicine, University of Salamanca; [3]Department of Statistics, University of Salamanca, Salamanca, Spain

Key words: asthma; genetics; haplotype; polymorphism; PTGDR.

María Isidoro-García
Molecular Medicine Unit
Faculty of Medicine
Campus Miguel Unamuno s/n
37008 Salamanca
Spain

Accepted for publication 5 November 2005

The allergic reaction could be considered as an inappropriate inflammation involving the activation of mast cells. The cells secret different mediators that result in the accumulation of Th2 cells, eosinophils and basophils. Among these mediators, prostaglandin D2 is the most abundantly produced cyclooxygenase metabolite of arachidonic acid in response to environmental allergens, and it has been proposed as a mast cell activation marker in asthma (1). Prostaglandin D2 exerts its biological actions through the D prostanoid receptor (PTGDR). The gene encoding this receptor is located in chromosome 14q22 (2) and the corresponding protein is a G protein-couple receptor of 359 AA with a molecular weight of 40.276 kDa (2). Prostaglandin D2 receptor has recently been associated with asthma and atopy because of its genetic location (3, 4) and because of the results obtained in functional studies performed in mouse models. It has been demonstrated that the airway inflammatory response to allergen is highly inhibited when *PTGDR* is deleted (5).

The association between asthma and *PTGDR* promoter polymorphisms has been recently reported (6). It has been shown that the capacity of the regulatory regions located in the *PTGDR* promoter to bind transcription factors, and so to control the PTGDR expression, is modified by the presence of the analyzed polymorphisms. These changes in PTGDR expression determine differences in asthma susceptibility. These findings also point PTGDR as a putative therapeutic target. In this sense, new PTGDR antagonists should be considered (7). The relevance of recognizing which genetic polymorphism is associated with which aspect of clinical phenotypes has been recently highlighted (8). It has been suggested that correlation with severity of asthma might be helpful for making decisions regarding therapy. Considering the lack of information about *PTGDR* polymorphisms with regard to asthma phenotypes, our purpose was to study their association with the development of asthma in a population of patients with well-characterized asthma phenotype.

Methods

Study populations

This study was performed in a Spanish population of 197 Caucasian unrelated individuals from the Allergy Department of the University Hospital of Salamanca. The study was performed after the

approval and following the recommendations of the Ethical Committee of the University Hospital of Salamanca, including informed written consent in all cases.

A total of 118 patients with physician-diagnosed asthma were recruited. They were included in the study if fulfilled the following criteria: at least two symptoms consistent with asthma (cough, wheeze and dyspnoea); either bronchial hyperreactivity (BHR), defined by a positive methacholine test, or a positive bronchodilator test; and absence of other pulmonary disorders. Lung function was measured by spirometry following the American Thoracic Society (ATS) criteria (9). Severity of asthma was classified according to Global Initiative for Asthma (GINA) guidelines (10).

Seventy-nine healthy individual were enrolled as controls when meeting all the following criteria: (i) no symptoms or history of asthma or other pulmonary diseases; (ii) no symptoms or history of allergy; (iii) negative skin prick tests to a battery of common aeroallergens (<1 mm wheal greater than saline); (iv) absence of first degree relatives with a history of asthma or atopy.

Skin prick testing was performed following The European Academy of Allergy and Clinical Immunology (EAACI) allergen standardization and skin test recommendations (11) with a battery of common aeroallergens that included *Dermatophagoides pteronisynuss*, *Dermatophagoides farinae*, *Lepidoglyphus destructor*, *Tyrophagus putrescentiae*, *Euroglyphus maynei*, *Acarus siro*, *Glycyphagus domesticus*, mix of grasses, mix of trees, *Parietaria judaica*, *Chenopodium album*, *Artemisa vulgaris*, *Plantago ovata*, *Olea europaea*, *Alternaria alternata*, *Cladospporium herbarum*, *Penicillium notatum*, *Aspergillus fumigatus*, dog, cat, hamster, horse and rabbit dander and cockroach (ALK-Abelló, Madrid, Spain). Histamine 10 mg/ml was used as positive control and saline was used as negative control. Before skin testing, antihistamines were discontinued according to published guidelines. The allergic status was based on the positive skin test to at least one allergen. Skin tests were considerer positive if at least one wheal reaction of >3 mm of diameter after subtraction of the negative control was observed. Total serum IgE levels were measured by a fluorenzymeimmunoassay (Pharmacia Cap System, Pharmacia, Uppsala, Sweden), following the manufacturer's instructions.

Genotype and haplotype analysis

For the genotype study, genomic DNA purification from total blood was performed with the *DNA Extraction Kit* (Genedan, S.L., Barcelona, Spain). A 640 bp fragment comprising the three single nucleotide polymorphisms' (SNP) positions ($-549T>C$, $-441C>T$ and $-197T>C$) from *PTGDR* promoter region was amplified by polymerase chain reaction (PCR) in a MWG-BIOTHECH thermal cycler (Biothech, Ebersberg, Germany). The sequences of the upstream and downstream primers employed in these amplifications were 5′-CTCAGTTTCCTCGCCTATGC-3′ and 5′-ACCCCTGG AAGCCTACAACTGCAT-3′, respectively.

The reaction mixtures were performed in 25 μl final volume reactions including 20 ng of genomic DNA, 1.25 pmol of each primer and 12.5 μl of a commercial PCR Master Mix (Promega, Madison, WI) containing Taq polimerase, dNTPs and $MgCl_2$. Cycling conditions included one cycle of 95°C for 5 min; 40 cycles at 94°C for 1 min, 60°C for 1 min and 72°C for 1 min; and a final additional extension period at 72°C for 10 min. To avoid contaminations negative controls without genomic DNA were included in each PCR reaction. All fragments were visualized using ethidium bromide on a 2% agarose gel. To clean up the PCR products, GENECLEAN Turbo kit (Q-BIOgene, Irvine, CA, USA) was used. The amplicons were sequenced in a 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using the primers previously employed in PCR amplification. Chromas 2.3 (Technelysium Pty. Ltd 1998–2004, Tewantin, Australia) was used to aligned and view the resulting chromatograms. The Genebank accession number for the reference genomic sequence used for *PTGDR* alignments was AL355833.4; GI: 13990340.

Laboratory procedures were performed following the European Molecular Genetics Quality Network best practice guidelines (12, 13). Specific quality measures were taken in all laboratory procedures beginning with sample reception and storage. Steps to minimize the risk of contamination during DNA extraction process were taken. The number of tube-to-tube transfers was minimized and ready-made solutions from commercial manufacturers were routinely employed. Separate pre- and post-PCR areas were established to minimize risk of contamination and safety cabinets and filter tips for pipettes were utilized. Normal control samples, molecular weight markers and negative controls were used in the process. The sequencing method was chosen to genotype samples because of its liability. Control and patients were not genotyped in separated batched. The analysis was performed blindly with respect to case–control status. All cases were doubling genotyped with 100% concordant results.

Statistical analysis

The allele and genotype frequencies in patients were compared with the control non-asthmatic population, for case–control studies. Hardy–Weinberg equilibrium was evaluated for each SNP by χ^2-test, Pearson's *P*-value is provided. The dichotomous variables were analyzed using χ^2-test, Fisher exact test and Monte Carlo simulation (after 10^4 simulations) on contingency tables for comparisons of the distribution of categorical variables. ANOVA was used to compare continuous variables across the levels of each genotype. Immunoglobulin E (IgE) levels were transformed to $\log_{10}$ values to produce a normal distribution for statistical analysis and analyzed by ANOVA. The effects of multiple covariates were modelled by logistic regression. Sex and age were included as potential covariates in multivariate analysis. A *P*-value of <0.05 was considered statistically significant. Haplotypes were estimated by expectation-maximization based algorithm using SNP analyzer web-based (14). The χ^2-statistics were derived from simple 2×2 tables based on frequency of each haplotype *vs* all other combined, for differences in haplotype frequencies between the case and control groups. Haplotype analysis was simultaneously performed considering all haplotypes with a frequency of $>1\%$ among either patients or controls, using Monte Carlo simulation test, normal χ^2-test and odds ratio test. Statistical analyses were performed by using software SPSS, version 12.0 (Chicago, IL, USA), and the SHEsis software platform (15).

Table 1. Phenotypic characteristics of the study population

Characteristic	Controls	Patients	*P*-value
Number of subjects	79	118	
Age ± SD (years)	40.2 ± 17.5	31.9 ± 16.9	0.001
Sex (*n*)			
Female	54	73	0.37
Male	25	45	
Log IgE			
Geometric mean	1.52	2.29	<0.0001
95% Confidence interval	1.39–1.66	2.18–2.41	

13989995, 2006, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2006.01025.x by Universidad De Salamanca, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Results

The clinical characteristics of the studied subjects are sumarized in Table 1. Control individuals were older to permit a longer period for an asthma diagnosis to be made. The distribution by sex was similar in both groups. IgE levels were higher in the group of patients than in controls.

Allele and genotype analysis

Allele and genotype frecuencies are presented in Table 2. We did not find statistically significant association between asthma phenotype and the allelic distribution of polymorphisms −549T > C (Fisher's *P*-value = 0.30) and −441C > T (Fisher's *P*-value = 0.41). Neither the genotype distribution of −549T > C nor −441C > T polymorphisms seemed to be associated with asthma phenotype (Fisher's *P*-value = 0.51 and 0.37, respectively) (Table 2). Nevertheless, the genotype distribution of the −197T > C polymorphism was significantly associated with asthma [Fisher's *P*-value = 0.007, Monte Carlo *P*-value (after 10^4 simulations) = 0.004] (Table 2).

Multivariate analysis of the genotypes adjusted for age and sex confirmed this association with an increased risk of asthma (OR, 3.06; 95% CI, 1.28–7.32; *P*-value = 0.012) for the comparison of TT with TC. Genotype distributions for the SNPs were in Hardy–Weinberg equilibrium (*P*-value > 0.05) except for −197T > C polymorphism in controls (Table 2). Samples were regenotyped and all these results were replicated according to previously reported recommendations (16).

Table 2. Genotype and allele frequencies of −549T>C, −441C>T and 197T>C polymorphisms, according to asthma phenotype

		Genotype			Allele		
Phenotype	*n*	TT	TC	CC	T	C	HWE *P*-value
−549T>C							
Controls	79	0.23	0.56	0.21	0.51	0.49	0.31
All asthma	118	0.19	0.52	0.29	0.45	0.55	0.64
Allergic asthma	90	0.20	0.52	0.28	0.46	0.54	0.63
		CC	TC	TT	C	T	
−441C>T							
Controls	79	0.57	0.39	0.04	0.77	0.23	0.40
All asthma	118	0.65	0.30	0.05	0.80	0.20	0.45
Allergic asthma	90	0.66	0.29	0.05	0.80	0.20	0.36
		TT	TC	CC	T	C	
−197T>C							
Controls	79	0.85	0.10	0.05	0.90	0.10	<0.05
All asthma*	118	0.74	0.25	0.01	0.86	0.14	0.36
Allergic asthma†	90	0.71	0.28	0.01	0.85	0.15	0.40

HWE, Hardy–Weinberg equilibrium.
*Fisher's *P*-value = 0.007 Monte Carlo *P*-value (after 10^4 simulations) = 0.004.
†Fisher's *P*-value = 0.007 Monte Carlo *P*-value (after 10^4 simulations) = 0.005.

Table 3. Haplotype frequencies of *PTGDR*

Haplotype*	Controls ($n = 79$)	Asthma ($n = 118$)	Allergic asthma ($n = 90$)
CCT	0.39	0.43	0.41
TCT	0.27	0.25	0.25
TTT	0.22	0.19	0.19
CCC	0.09	0.12†	0.13‡

*The order of the SNPs in the haplotype was −549T>C, −441C>T and −197T>C. Haplotypes with a frequency of >1% among either controls or patients were included.
†Fisher's *P*-value = 0.027 comparing the CCC haplotype against all other haplotypes.
‡Fisher's *P*-value = 0.019 comparing the CCC haplotype against all other haplotypes.

In our population, the association of the −197T > C polymorphism with asthma was also identified in patients with allergic asthma [Fisher's *P*-value = 0.007, Monte Carlo *P*-value (after 10^4 simulations) = 0.005] (Table 2). No association was observed with IgE levels.

Haplotype analysis

The different promoter polymorphism combinations gave rise to the following haplotype variants CCT, TCT, TTT, CCC, TCC, TTC, CTT (promoter positions −549, −441 and −197). The first four were more common. Haplotype frequences among patients and controls are shown in Table 3.

Although in the haplotype analysis, we did not found any association between the global haplotype distribution and the asthma phenotype, a separate analysis of the CCC haplotype frequencies against all other haplotypes showed a significant difference *P*-value = 0.027 (OR, 2.48; 95% CI, 1.10–5.59). This haplotype was more common in patients with asthma (0.76) than in controls (0.24), particularly in patients with allergic asthma (OR, 2.79; 95% CI, 1.21–6.46; *P*-value = 0.019). No differences were observed for the rest of haplotypes individually considered.

Diplotype analysis

In the diplotype analysis, there was a difference in diplotype frequencies comparing patients with asthma and controls (*P*-value = 0.028) (Table 4); this was specifically for the CCT CCC diplotype (−549CC, −441CC and −197TC). A separate analysis of this diplotype frequencies against all other diplotypes showed a specific association of CCT CCC diplotype with asthma (*P*-value < 0.0001; OR, 1.15; 95% CI, 1.07–1.23). This diplotype was also found to be associated with allergic asthma (*P*-value < 0.0001; OR, 1.16; 95% CI, 1.07–1.26).

It is very interesting to notice that all patients who carried this diplotype developed asthma; this diplotype was not present in any of the control individuals. This diplotype was unambiguous.

13989995, 2006, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2006.01025.x by Universidad De Salamanca, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 4. Diplotype frequencies of *PTGDR*

Diplotype*	Controls ($n = 79$)	Asthma ($n = 118$)	Allergic asthma ($n = 90$)
CCT CCT	0.16	0.16	0.14
CCT TCT	0.23	0.26	0.26
CCT TTT	0.26	0.16	0.15
CCT CCC	0.00	0.13†	0.14‡
TCT TCT	0.09	0.04	0.04
TCT TTT	0.09	0.09	0.09
TCT CCC	0.05	0.06	0.07
TTT TTT	0.04	0.04	0.04
TTT CCC	0.03	0.05	0.06
CCC CCC	0.05	0.01	0.01

*The order of the SNPs in the haplotype was −549T>C, −441C>T and −197T>C. Diplotypes with a frequency of >1% among either controls or patients were included.

†*P*-value < 0.0001 comparing CCTCCC diplotype against all other haplotypes.

‡*P*-value < 0.0001 comparing CCTCCC diplotype against all other haplotypes.

Discussion

The genetic location of PTGDR gene has been linked to asthma and atopy (3, 4). This receptor has been probed to be necessary for the development of the asthma phenotype in a mouse model in which the airway inflammatory response to allergen was highly inhibited when *PTGDR* was deleted (5). In our population, a trend of association between −197T > C polymorphism and asthma phenotype was detected. This association was also confirmed in patients with allergic asthma.

Recently, Oguma et al. (6) found that −549T > C, −441C > T and −197T > C SNPs occur in regions of the PTGDR promoter that bind transcription factor C/EBPβ and members of the Sp and GATA families. Therefore, the presence of these SNPs influences gene expression and determine differences in asthma susceptibility. They reported an association between −549T > C and −441C > T polymorphisms and asthma, but they did not mention association among asthma phenotype and the −197T > C polymorphism in their populations. This could be because of true variation in the underlying association between this genotype and asthma. The allele could be in linkage disequilibrium with a different allele in both populations or allelic heterogeneity could exist between different populations. This would be more likely when the variant was uncommon. The association could also be modified by other genetics or environmental factors that could vary between Oguma's American population and our European population. In addition, different criteria were used to phenotypic classification and Oguma's population was mainly limited to moderate to severe asthma (17), whereas our population presented a broader spectrum of asthma severity. Finally, our criteria for control selection were more astringent. All these reasons could have contributed to explain such discrepancies, although biases variation between studies or effects from multiple comparisons can also be considered. To minimize these effects, strict measures of quality control were taken, both in laboratory procedures and in statistical analysis. Any case, the true model of genetic susceptibility for diseases such as asthma and atopy is complex (18, 19). In this sense, it has been recently highlighted the lack of information about *PTGDR* variants association with asthma in other populations (7). To the best of our knowledge, this is the first study performed in Europe about *PTGDR* in patients with asthma.

As long as combinations of variants had larger functional effects than individual variants, and might better explain susceptibility to asthma, we analyzed the haplotype combinations in our population. We detected an association between CCC haplotype and asthma. Oguma et al. (6) analyzed the effects of the haplotype combinations of these SNPs in the increase or decrease of the receptor transcription, which determines higher or lower asthma susceptibility (7, 20). They showed that the CCC haplotype was associated with a significantly higher *PTGDR* gene expression followed by the CCT haplotype. They also detected that patients who had at least one copy of the high-transcriptional efficiency haplotype (CCC) were more likely to have asthma. Inversely, patients with TCT (low transcriptional efficiency) were less likely to have asthma. We have not detected the protector effect of TCT haplotype, in our population.

We have also identified differences in diplotype combinations between patients with asthma and controls. A specific association of the diplotype combination CCT CCC and asthma was found. It is very interesting to notice that this specific diplotype is a combination of the two high-transcriptional efficiency haplotypes reported (6). In our population, all patients who carried the CCT CCC diplotype developed asthma; this diplotype was not present in any of the control individuals. The control sample size is derived from the restrictive criteria for control selection in our study. With this sample, we found significant statistical differences among the diplotype frequencies of patients and controls (Fisher's *P*-value < 0.0001). As this is the first study of this diplotype in our population, there is no data on its frequency, thus we could not reliably calculate the number of controls that were required to show any significant association. Considering as expected frequency the reported by Oguma et al. (6), the alpha error for the sample size of our control group would be < 0.05, and the statistical power 85%. Due to the differences between Oguma's population and ours, we could also consider as expected frequency, the frequency detected in our asthmatic patients; in this case, the alpha error estimated for the sample size of our controls would be < 0.05, and the statistical power 92%. In addition, this diplotype was unambiguous. Our *in vivo* results would confirm for the first time what was demonstrated *in vitro* about higher-transcriptional efficiency haplotypes in asthma.

We want to point out that the association of this CCT CCC diplotype combination was especially observed in

13989995, 2006, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2006.01025.x by Universidad De Salamanca, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

patients with allergic asthma. In this sense, it has been recently reported that the activation of the prostaglandin D2 receptor increases allergic inflammation in mouse, supporting the hypothesis that the prostaglandin D2 receptor might play a critical role in allergic diseases (21). Our results would be also in agreement with a previous study (5) in which the prostanoid DP receptor was demonstrated to be necessary for the occurrence of allergic sensitization in a mouse model of asthma.

Genetic association studies for detection of genetic variants contributing to complex outcomes must be considered with caution because many factors may be influencing in positive results. These association studies need careful classification of phenotypes and application of quality control in the performance of laboratory procedures and statistical analysis (22, 23) although true heterogeneity in gene-disease associations may be found. In this study, the asthma phenotype was classified following restrictive criteria. To guarantee the quality of laboratory procedures, SNPs were closely scrutinized and retyped to ensure that they were genotyped correctly. Patients and controls were not genotyped in separated batches and coding of samples was not separated for controls and patients, so analysis was performed doubled blinded (16). All measures were taken following European Molecular Genetics Quality Network (EMQN) good practice guidelines. To limit type I error, the number of empirical analyses should be limited and very stringent significant levels must be considered to assure reproducibility. Inconsistencies in association studies may well result from the fact that many analysis are focused only on one SNP and that penetrance of the alleles may be influence by other factors. The functional significance of a given polymorphism may only be evident in a specific setting of additional SNPs in the same or different genes (24). It has been suggested (6) that large-scale studies of *PTGDR* variants in population-based samples will be necessary to quantify the proportion of asthma risk that is accounted for by these variants in populations with the disease. It has also been pointed out that most identified polymorphisms do not appear to carry risks that would merit their use in a clinical setting, but combinations of genetic polymorphisms may be much more informative (25).

Without excluding other functional variants in linkage disequilibrium with the ones reported, we identified a more specific genetic variant of *PTGDR* that could be associated with asthma. As previously proposed (6), the study of *PTGDR* combination effects in different populations might contribute to improved risk models that may have useful predictive power.

Acknowledgments

This work was partially supported by a grant of the Fundación de la Sociedad Española de Alergología e Inmunología Clínica.

References

1. Murray JJ, Tonnel AB, Brash AR, Roberts LJ, Gosset P, Workman R et al. Release of prostaglandin D2 into human airways during acute antigen challenge. N Engl J Med 1986;**315**:800–804.
2. Boie Y, Sawyer N, Slipetz DM, Metters KM, Abramovitz M. Molecular cloning and characterization of the human prostanoid DP receptor. J Biol Chem 1995;**270**:18910–18916.
3. Daniels SE, Bhattacharrya S, James A, Leaves NI, Young A, Hill MR et al. A genome-wide search for quantitative trait loci underlying asthma. Nature 1996;**383**:247–250.
4. Mansur AH, Bishop DT, Markham AF, Morton NE, Holgate ST, Morrison JF. Suggestive evidence for genetic linkage between IgE phenotypes and chromosome 14q markers. Am J Respir Crit Care Med 1999;**159**:1796–1802.
5. Matsuoka T, Hirata M, Tanaka H, Takahashi Y, Murata T, Kabashima K et al. Prostaglandin D2 as a mediator of allergic asthma. Science 2000;**287**:2013–2017.
6. Oguma T, Palmer LJ, Birben E, Sonna LA, Asano K, Lilly CM. Role of prostanoid DP receptor variants in susceptibility to asthma. N Engl J Med 2004;**351**:1752–1763.
7. Lilly CM, Palmer LJ. The role of prostaglandin d receptor gene in asthma pathogenesis. Am J Respir Cell Mol Biol 2005;**33**:224–226.
8. Morita H, Nagai R. Prostanoid DP receptor variants and asthma. N Engl J Med 2005;**352**:837–838.
9. American Thoracic Society. Standards for the diagnosis and care of patients with chronic obstructive pulmonary disease. American Thoracic Society. Am J Respir Crit Care Med 1995;**152**(5 Pt 2):S77–S121.
10. Global Initiative for Asthma. Global Strategy for Asthma Management and Prevention. NHLBI/WHO workshop report. NIH Publication number 02–3659, 2002.
11. Dreborg S, Backmann A, Basomba A, Bousquet J, Diegues P. Skin tests used in type I allergy testing position paper. Subcommittee on Skin Tests of the European Academy of Allergology and Clinical Immunology. Allergy 1989;**44**(Suppl. 10):1–59.
12. Muller CR. Quality control in mutation analysis: the European Molecular Genetics Quality Network (EMQN). Eur J Pediatr 2001;**160**:464–467.
13. Patton SSS. Draft Best Practice Guidelines for Laboratory Internal Quality Control. European Molecular Genetics Quality Network 2002; Homepage for EMQN: http://www.emqn.org (Accessed 5 November 2005).
14. Workbench J, Bonghee S, Yangseok K. SNP Analyzer: Web Based for the SNPs Analysis. Genome Inform 2003;**14**:591–592.
15. Shi YY, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 2005;**15**:97–98.

13989995, 2006, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2006.01025.x by Universidad De Salamanca, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

16. Xu J, Turner A, Little J, Bleecker ER, Meyers DA. Positive results in association studies are associated with departure from Hardy–Weinberg equilibrium: hint for genotyping error? Hum Genet 2002;**111**:573–574.
17. Oguma T, Palmer L, Birben E, Sonna L, Asano K, Lilly C. Prostanoid DP receptor variants and asthma. N Engl J Med 2005;**352**:837–838.
18. Hoffjan S, Nicolae D, Ober C. Association studies for asthma and atopic diseases: a comprehensive review of the literature. Respir Res 2003;**4**:14.
19. Ober C. Perspectives on the past decade of asthma genetics. J Allergy Clin Immunol 2005;**116**:274–278.
20. Lilly CM. Diversity of asthma: evolving concepts of pathophysiology and lessons from genetics. J Allergy Clin Immunol 2005;**115**(Suppl. 4):S526–S531.
21. Spik I, Brenuchon C, Angeli V, Staumont D, Fleury S, Capron M et al. Activation of the prostaglandin D2 receptor DP2/CRTH2 increases allergic inflammation in mouse. J Immunol 2005;**174**:3703–3708.
22. Colhoun HM, McKeigue PM, Davey SG. Problems of reporting genetic associations with complex outcomes. Lancet 2003;**361**:865–872.
23. Gomes I, Collins A, Lonjou C, Thomas NS, Wilkinson J, Watson M et al. Hardy-Weinberg quality control. Ann Hum Genet 1999;**63**(Pt 6):535–538.
24. Risma KA, Wang N, Andrews RP, Cunningham CM, Ericksen MB, Bernstein JA et al. V75R576 IL-4 receptor alpha is associated with allergic asthma and enhanced IL-4 receptor function. J Immunol 2002;**169**:1604–1610.
25. Cookson W, Moffatt M. Making sense of asthma genes. N Engl J Med 2004;**351**:1794–1796.

13989995, 2006, 5, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/j.1398-9995.2006.01025.x by Universidad De Salamanca, Wiley Online Library on [15/07/2024]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License