BMB
reports

Association of *PTGER* gene family polymorphisms with aspirin intolerant asthma in Korean asthmatics

Byung-Lae Park[1], Se-Min Park[2], Jong-Sook Park[2], Soo-Taek Uh[2], Jae-Sung Choi[2], Yong-Hoon Kim[2], Mi-Kyeong Kim[3], Inseon S. Choi[4], Byoung-Whui Choi[5], Sang-Heon Cho[6], Chein-Soo Hong[7], Yong-Won Lee[7], Jae-Young Lee[8], Choon-Sik Park[2,] & Hyoung-Doo Shin[1,9,*]*

[1]Department of Genetic Epidemiology, SNP Genetics, Inc., Seoul 153-803, [2]Genome Research Center for Allergy and Respiratory Medicine, Soonchunhyang University Hospital, Bucheon 420-021, [3]Division of Internal Medicine, Chungbuk National University, College of Medicine, Cheongju 361-711, [4]Department of Allergy, Chonnam National University Medical School and Research Institute of Medical Sciences, Gwangju 501-757, [5]Division of Pulmonology and Allergy, Department of Internal Medicine, Chung-Ang University Yongsan Hospital, Seoul 140-757, [6]Department of Internal Medicine and Institute of Allergy and Clinical Immunology, Seoul National University College of Medicine, Seoul 110-744, [7]Division of Allergy and Immunology, Institute of Allergy, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-752, [8]Pulmonary, Allergy & Critical Care Medicine, Hallym University College of Medicine, Chuncheon 200-702, [9]Department of Life Science, Sogang University, Seoul 121-742, Korea

Aspirin-intolerant asthma (AIA) is characterized by severe asthmatic attack after ingestion of aspirin and/or non-steroidal anti-inflammatory drugs. In this study, we investigated the relationship between *Prostaglandin E2 receptor (PTGER)* gene family polymorphisms and AIA in 243 AIA patients and 919 aspirin-tolerant asthma (ATA) controls of Korean ethnicity in two separate study cohorts. After genotyping 120 SNPs of the *PTGER* gene family for the 1st cohort study, four SNPs in *PTGER1*, ten in *PTGER3*, six in *PTGER3*, and a haplotype of *PTGER2* showed association signals with decreased or increased risk of AIA. Among the positively associated SNPs, one in *PTGER1* and four in *PTGER3* were analyzed in the 2nd cohort study. The results show that *rs7543182* and *rs959* in *PTGER3* retained their effect, although no statistical significance was retained in the 2nd cohort study. Our findings provide further evidence that polymorphisms in *PTGER3* might play a significant role in aspirin hypersensitivity among Korean asthmatics. [BMB reports 2010; 43(6): 445-449]

INTRODUCTION

Acetyl salicylic acid (ASA; aspirin)-intolerant asthma (AIA) is a distinct clinical condition characterized by the 'aspirin triad' syndrome of aspirin hypersensitivity, eosinophilic rhinosinusitis, nasal polyposis, and bronchial asthma, and is known to affect 10-20% of adult asthmatic patients (1-7). AIA is due to the development of bronchoconstriction in asthma patients following ingestion of aspirin or other non-steroidal anti-inflammatory drugs (NSAIDs). Although the underlying mechanisms of AIA have not been elucidated completely, arachidonic acid metabolites such as prostaglandins (PGs), leukotrienes (LTs), and thromboxane (TBX) are reportedly involved in the pathogenesis of this asthma phenotype (8-10). Anti-inflammatory PGs are one of the various types of oxygenated metabolites generated by the cyclooxygenase (COX) pathway that function to reduce the level of PGE2, the main COX2 metabolite (11-13). The cyclooxygenase theory is widely accepted as responsible for the pathogenesis of AIA. Inhibition of COXs by NSAIDs in the respiratory tract alters arachidonic acid metabolism, leading to reduction of PGE2 and alteration of AIA pathogenesis (9-11, 14, 15). Therefore, the overproduction or underproduction of critical mediators of arachidonic acid metabolism, such as PGs, probably accounts for disease susceptibility (2).

Prostaglandin E2 (PGE2), an arachidonic acid metabolite, is a key factor in the generation of abnormal pain sensations incited by inflammation (16). PGE2 is synthesized and secreted by diverse cell types and plays an important role in balancing the Th1/Th2 immune responses (17, 18). PGE2 exerts its biological effects by binding to four different G protein-coupled receptors encoded by the following separate genes: *PTGER1* (MIM# 176802), *PTGER2* (MIM# 176804), *PTGER3* (MIM# 176806), and *PTGER4* (MIM# 601586). These four receptors shows different tissue distribution and physiological functions and work in different signaling pathways (19-21). The role of *PGE2* in the pathogenesis of AIA was investigated by focusing on reduced PGE2 release caused by aspirin-induced cyclooxygenase inhibition (11, 14, 22).

Although a recent study suggested that the role of *PGE2* in

*Corresponding author. Choon-Sik Park, Tel: 82-32-621-5105; Fax: 82-32-621-5023; E-mail: mdcspark@unitel.co.kr, Hyoung-Doo Shin, Tel: 82-2-705-8615; Fax: 82-2-2026-4299; Email: hdshin@sogang.ac.kr

Received 1 February 2010, Accepted 7 May 2010

Keywords: Aspirin intolerant asthma, Asthma, Prostaglandin E receptor(s), Single nucleotide polymorphism

the pathogenesis of asthma is independent of aspirin intolerance (23), others have shown that genetic variations in *PGE2* receptors are strongly associated with AIA pathogenesis (24, 25).

Therefore, to replicate the effects of these genetic variations, we genotyped 120 polymorphisms of the *PTGER* gene family in a large case-control study of a Korean population and assessed their risk of AIA (n = 1,162).

RESULTS AND DISCUSSION

In the present study, a total of 1,162 subjects were recruited for the asthma cohort, the clinical characteristics of which are summarized in Table 1. Significant differences in mean age, prevalence of smoking, and body mass index (BMI) were found between the AIA and the ATA groups ($P < 0.05$). Forced vital capacity in the first second (FVC_1), forced expiratory volume in the first second (FEV_1), and log [20% fall in FEV1 (PC20)] were significantly different between AIA and ATA groups in the 2nd study. The mean aspirin-induced decline in FEV_1 was lower in the 2nd study than in the 1st study due to a larger number of AIA-intermediate subjects in the 2nd study (34.3% of AIA-intermediates in 1st study and 54.7% in 2nd study). Furthermore, an aspirin-induced decline in FEV1 from -15% to 82% was observed among the subjects.

For the 1st study, we scrutinized candidate gene variants in four *PTGER* genes from databases of NCBI dbSNP (build 36). One hundred and twenty variants, including 2 SNPs in *PTGER1*, 12 in *PTGER2*, 103 in *PTGER3*, and 3 in *PTGER4*, were successfully genotyped using the Illumina Golden Gate genotyping system in 137 AIA and 268 ATA subjects of Korean ethnicity. The overall call rate for all SNPs was 99.80%. The minor allele frequencies (MAFs) of these 120 SNPs in each AIA and ATA group are summarized in supplementary Table 1. The distributions of all loci were in Hardy-Weinberg equilibrium ($P > 0.01$) except *rs11209710* and *rs7543182* (P = 0.005 and 0.007, respectively). Haplotypes of the four PTGER receptor genes were constructed, and PTGER3 was parsed into three haplotype blocks with each block having a strong LD spine (Data not shown). There were three common haplotypes in *PTGER1*, six in *PTGER2*, five in block 1 of *PTGER3*, three in block 2 of *PTGER3*, five in block 3 of *PTGER3*, and three in *PTGER4*. Multiple logistic regression models found that two SNPs and two haplotypes of *PTGER1* along with ten SNPs of *PTGER3* were marginally associated with intolerance to AIA, whereas six SNPs of *PTGER3* along with a haplotype of *PTGER2* showed marginal association with increased risk of AIA (Supplementary Table 2). Among the positively associated SNPs, one in *PTGER1* (*rs2241363*) and four in *PTGER3* (*rs6424414, rs516647, rs7543182 and rs959*) were selected due to their LD status for a 2nd replication study of 106 AIA and 651 ATA subjects. Statistical analysis revealed that *rs7543182* and *rs959* in *PTGER3* retained their effects in the 2nd cohort. That is, these two polymorphisms retained their susceptibility to aspirin intolerance, although there was no longer any statistical significance (Table 2). However, *rs2241363* in *PTGER1* along with *rs6424414* and *rs516647* in *PTGER3* failed to replicate its effect and statistical significance in the 2nd cohort.

A recent study using PTGER3-knockout mice showed that the relationship between PGE2 and asthma is unrelated to aspirin intolerance (23), whereas genetic variations in PGE2 receptors may be an important genetic factor in the pathogenesis of AIA in Japanese individuals (25). In the Korean population, polymorphisms in the *PTGER2, PTGER3,* and *PTGER4* genes

Table 1. Clinical profiles of study subjects

Clinical profile	1st study		2nd study		All	
	AIA	ATA	AIA	ATA	AIA	ATA
Number of subjects	137	268	106	651	243	919
Age of first medical examination [mean (range)]*	43.73 (17-72)	46.77 (15-77)	37.48 (17-72)	44.43 (11-78)	41.00 (17-72)	45.11 (11-78)
Sex (male/female)	48/89	88/180	47/59	255/396	95/148	343/576
Current smoker (%)*	24.9%	32.8%	22.7%	28.3%	23.9%	29.6%
BMI (kg/m^2)*	23.71 ± 3.05	24.62 ± 3.49	23.07 ± 3.14	24.20 ± 3.50	23.43 ± 3.10	24.32 ± 3.50
Fall rate (%)*	24.81 ± 15.79	3.48 ± 4.52	10.63 ± 11.72	4.19 ± 5.05	21.31 ± 16.07	3.96 ± 4.90
Log [Blood eosinophil (%)]	0.76 ± 0.30	0.75 ± 0.32	0.66 ± 3.12	0.69 ± 0.32	0.72 ± 0.31	0.70 ± 0.32
FVC1%, predicted	83.55 ± 18.50	85.29 ± 18.74	90.329 ± 15.24	83.26 ± 17.68	86.26 ± 17.55	83.86 ± 18.00
FEV1%, predicted	82.99 ± 19.74	83.44 ± 21.50	88.76 ± 17.33	83.92 ± 18.36	85.31 ± 18.99	83.78 ± 19.32
Log [PC20, methacholine (mg/ml)]	0.49 ± 0.45	0.46 ± 0.32	0.76 ± 0.51	0.64 ± 0.45	0.59 ± 0.49	0.58 ± 0.42
Log [Total IgE (IU/ml)]	2.22 ± 0.52	2.18 ± 0.61	2.23 ± 0.51	2.16 ± 0.66	2.22 ± 0.52	2.17 ± 0.64
Positive rate of specific IgE (D.f., %)	36.8%	34.7%	46.3%	40.7%	39.6%	38.8%
Positive rate of specific IgE (D.p., %)	43.6%	43.3%	57.4%	48.4%	47.6%	46.8%
Positive rate of skin test (%)	40.2%	44.4%	37.7%	37.5%	39.1%	39.5%

Asterisks mean $P < 0.05$.

Table 2. Logistic analysis of PTGER gene family polymorphisms with the risk of aspirin intolerant asthma controlling age, sex, BMI and smoking status in aspirin tolerant asthma subjects

Gene	rs#	Stage	MAF		Co-dominant		Dominant		Recessive	
					OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)	P
		First stage	AIA (n = 137)	ATA (n = 268)						
PTGER1	rs2241363		0.361	0.427	0.77 (0.56-1.05)	0.09	0.96 (0.62-1.49)	0.86	0.37 (0.19-0.73)	0.004
PTGER3	rs6424414		0.460	0.498	0.85 (0.64-1.14)	0.28	1.16 (0.72-1.86)	0.55	0.53 (0.31-0.89)	0.02
	rs516647		0.449	0.481	0.87 (0.65-1.16)	0.34	1.18 (0.74-1.88)	0.49	0.53 (0.31-0.90)	0.02
	rs7543182		0.303	0.252	1.45 (1.01-2.09)	0.04	1.51 (0.99-2.31)	0.06	1.72 (0.65-4.55)	0.28
	rs959		0.463	0.394	1.43 (1.03-1.99)	0.03	1.39 (0.87-2.22)	0.17	1.89 (1.06-3.38)	0.03
		Replication	AIA (n = 106)	ATA (n = 651)						
PTGER1	rs2241363		0.400	0.403	0.96 (0.71-1.30)	0.78	1.11 (0.71-1.74)	0.64	0.71 (0.38-1.30)	0.27
PTGER3	rs6424414		0.519	0.455	1.28 (0.95-1.73)	0.11	1.56 (0.94-2.59)	0.08	1.24 (0.76-2.02)	0.40
	rs516647		0.514	0.451	1.27 (0.95-1.71)	0.11	1.54 (0.94-2.54)	0.09	1.24 (0.76-2.02)	0.39
	rs7543182		0.288	0.247	1.23 (0.88-1.73)	0.23	1.31 (0.86-1.99)	0.21	1.22 (0.52-2.86)	0.65
	rs959		0.429	0.375	1.28 (0.94-1.73)	0.12	1.76 (1.11-2.80)	0.02	0.91 (0.49-1.68)	0.77
		All	AIA (n = 243)	ATA (n = 919)						
PTGER1	rs2241363		0.378	0.410	0.87 (0.71-1.07)	0.19	1.04 (0.77-1.41)	0.80	0.53 (0.34-0.83)	0.005
PTGER3	rs6424414		0.486	0.467	1.06 (0.87-1.29)	0.59	1.33 (0.96-1.86)	0.09	0.85 (0.60-1.21)	0.37
	rs516647		0.477	0.460	1.05 (0.86-1.29)	0.61	1.31 (0.95-1.82)	0.10	0.85 (0.60-1.21)	0.36
	rs7543182		0.296	0.248	1.31 (1.04-1.66)	0.02	1.43 (1.07-1.90)	0.02	1.25 (0.68-2.29)	0.48
	rs959		0.448	0.381	1.36 (1.10-1.68)	0.005	1.66 (1.21-2.28)	0.002	1.27 (0.86-1.88)	0.24

The P values were obtained by logistic regression analysis, controlled for age (continuous value), sex (male = 0, female = 1), atopy status (non-atopy = 0, atopy = 1), BMI and smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2) as co-variables

are known to be associated with aspirin intolerance, whereas polymorphisms in *PTGER1* have not yet been reported to affect aspirin intolerance among asthmatics (24). Previous studies have shown that polymorphisms in *PTGER1* are unrelated with asthma and aspirin intolerance, although the inhibition of *PTGER1*-mediated signaling plays an important role in the effect of NSAIDs in pain reduction in an acetic acid model (24, 26, 27). However, the current study showed that *PTGER1 rs2241363* exhibited a protective effect with regards to AIA. To our knowledge, this is the first study demonstrating the importance of genetic variations in *PTGER1* to ones predisposition to aspirin intolerance.

PTGER3 is known to enhance the inflammatory response through coupling with PGE2. It is also involved in the suppression of allergic inflammation in an animal model of asthma (23). It has been reported that *PTGER3* polymorphisms are important genetic factors affecting the risk and severity of asthma as well as the pathogenesis of AIA (24, 26). Among two associated polymorphisms in *PTGER3, rs959* on the 3'UTR region was significantly associated with an increased risk of AIA in codominant and dominant models (P = 0.005 and 0.002, respectively). Although the mechanism of AIA associated with alternative genotypes in the 3' UTR is not totally understood, there are several possible explanations based on the role of the noncoding portion of the genome. It is known that mRNA UTRs are involved in many post-transcriptional regulatory pathways that control mRNA localization, stability, and translation efficiency. Moreover, the initiation of protein synthesis could be influenced by sequence elements in both the 5' and 3' UTRs. Therefore, post-transcriptional events play important yet not fully understood roles in the regulation of gene expression and cellular behavior. In fact, many of the cis-acting elements that affect translational regulation are located within the 3' UTR (28).

In summary, we examined the genetic association of four PGE2 receptors, including *PTGER1, PTGER2, PTGER3*, and *PTGER4*, with aspirin intolerance among Korean asthmatics (n=1,162). Although all signals were not retained after correction, our study suggests that polymorphisms in *PTGER3* might be important genetic factors in controlling aspirin intolerance among Korean asthmatics. Therefore, further biological and/or functional evidence is needed to confirm the associations in this study.

MATERIALS AND METHODS

Subjects

The subjects, all of whom were Korean, were recruited from the Asthma Genome Research Center, which is comprised of nine university hospitals in Korea. All patients were diagnosed by a physician and met the definition of asthma as set forth in the Global Initiative for Asthma (GINA) guidelines. All patients had a history of dyspnea and wheezing over the previous 12 months plus one of the following: 1) $>15\%$ increase in FEV1

or >12% increase plus 200 ml following inhalation of a short-acting bronchodilator, 2) <10 mg/ml PC20 methacholine, and 3) >20% increase in FEV1 following 2 weeks of treatment with inhaled steroids and long-acting bronchodilators. Twenty-four common inhalant allergens were used for a skin prick test (29). Total IgE was measured by the CAP system (Pharmacia Diagnostics, Uppsala, Sweden). Atopy was defined as having a wheal reaction equal to or greater than histamine or 3 mm in diameter. The asthmatic patients experienced no worsening of asthma and or tract infection during the 6 weeks preceding oral aspirin challenge (OAC). OAC was performed with increasing doses of aspirin using slightly modified methods (29, 30). Changes in FEV_1 were followed for 5 hours after the last aspirin challenge. Aspirin-induced bronchospasms, as reflected by rate (%) of FEV_1 decline, were calculated as the pre-challenge FEV_1 minus the post-challenge FEV_1 divided by the pre-challenge FEV_1. OAC reactions were categorized into 3 groups as follows: 20% or greater decrease in FEV_1 or 15% to 19% decrease in FEV_1 with naso-ocular or cutaneous reactions (aspirin intolerant asthma: AIA), 15% to 19% decrease in FEV_1 or naso-ocular or cutaneous reactions only (intermediate AIA: AIA-I), and 15% or less decrease in FEV_1 without naso-ocular or cutaneous reactions (aspirin tolerant asthma: ATA).

Peripheral venous blood was collected, and plasma was separated before and at the time of bronchospasm, or 2 hr after aspirin challenge. Peripheral blood mononuclear cells (PBMC) were separated in a Histopaque-1077 solution (1.077 g/ml, Sigma, St. Louis, MO). All subjects gave informed, written consent for participation in the study. The protocols were approved by the local ethics committees of each hospital.

SNP selection and genotyping

We selected polymorphic candidate SNPs for the 1st study from the National Center for Biotechnology Information (build 36), and then genotyped 137 AIA and 268 ATA subjects. For the 2nd study, additional subjects (106 AIA and 651 ATA) were employed. One SNP in *PTGER1* and four in *PTGER3* showing putative positive association in the 1st study were selected and genotyped. Genotyping in the 1st study was performed at the multiplex level using the Illumina Golden Gate genotyping system (31), and data quality was assessed by duplicate DNAs (n = 10). The genotype quality score for retaining data was set to 0.25. SNPs that could not satisfy the following criteria were excluded from the study: (i) a minimum call rate of 90%; (ii) no duplication error; (iii) Hardy-Weinberg equilibrium greater than P > 0.001. A total of 120 SNPs, including 2 SNPs in *PTGER1*, 12 SNPs in *PTGER2*, 103 SNPs in *PTGER3*, and 3 SNPs in *PTGER4*, were successfully genotyped. Genotyping probe information is presented in supplementary Table 3. Genotyping in the 2nd study was performed using TaqMan® (32) assay. Primer Express (Applied Biosystems) was used to design both the PCR primers and MGB TaqMan probes. One allelic probe was labeled with FAM dye while the other with fluorescent VIC dye. Genotyping probe information is presented in supplementary Table 4.

Statistics

We applied common measures of linkage disequilibrium to all pairs of biallelic loci: Lewontin's D' (|D'|) (33) and r^2. Haplotypes of each individual were inferred using PHASE algorithm (ver. 2.0) developed by Stephens *et al*. (34) The genotype and haplotype distribution were analyzed using logistic regression models using age (continuous value), gender (male = 0, female = 1), smoking status (non-smoker = 0, ex-smoker = 1, smoker = 2), atopy (absence = 0, presence = 1), and BMI as covariates. Differences in the rates of FEV_1 decline following aspirin challenge among the genotypes and haplotypes were examined using a linear regression model. The data were managed and analyzed using SAS version 9.1 (SAS Inc., Cary, NC).

Acknowledgements

This work was supported by a grant from the Korea Health 21 R&D Project, Ministry of Health, Welfare and Family Affairs, Republic of Korea (A010249), and was also supported by a grant from Korea Science and Engineering Foundation (KOSEF) funded by the Korean government (MEST) (No. 2009-0080157).

REFERENCES

1. Samter, M. and Beers, R. F., Jr. (1967) Concerning the nature of intolerance to aspirin. *J. Allergy* **40**, 281-293.
2. Szczeklik, A. and Stevenson, D. D. (2003) Aspirin-induced asthma: advances in pathogenesis, diagnosis, and management. *J. Allergy Clin. Immunol.* **111**, 913-921.
3. Jenkins, C., Costello, J. and Hodge, L. (2004) Systematic review of prevalence of aspirin induced asthma and its implications for clinical practice. *BMJ* **328**, 434.
4. Loewe, G., Slapke, J. and Kunath, H. (1985) Nasal polyposis, bronchial asthma and analgesic intolerance. *Rhinology.* **23**, 19-26.
5. Zeitz, H. J. (1988) Bronchial asthma, nasal polyps, and aspirin sensitivity: Samter's syndrome. *Clin. Chest. Med.* **9**, 567-576
6. Hedman, J., Kaprio, J., Poussa, T. and Nieminen, M. M. (1999) Prevalence of asthma, aspirin intolerance, nasal polyposis and chronic obstructive pulmonary disease in a population-based study. *Int. J. Epidemiol.* **28**, 717-722.
7. Berges-Gimeno, M. P., Simon, R. A. and Stevenson, D. D. (2002) The natural history and clinical characteristics of aspirin-exacerbated respiratory disease. *Ann. Allergy Asthma. Immunol.* **89**, 474-478.
8. Szczeklik, A. and Stevenson, D. D. (1999) Aspirin-induced asthma: advances in pathogenesis and management. *J. Allergy Clin. Immunol.* **104**, 5-13.
9. Picado, C. (2002) Aspirin-intolerant asthma: role of cyclo-oxygenase enzymes. *Allergy* **57(Suppl 72)**, 58-60.
10. Sampson, A., Holgate, S. Austen, K. F. and Szczeklik, A. (1998) Cyclo-oxygenase. *Thorax.* **53**, 719-720.
11. Pierzchalska, M., Szabo, Z., Sanak, M., Soja, J. and Szczeklik, A. (2003) Deficient prostaglandin E2 pro-

duction by bronchial fibroblasts of asthmatic patients, with special reference to aspirin-induced asthma. *J. Allergy Clin. Immunol.* **111**, 1041-1048.
12. Cowburn, A. S., Sladek, K., Soja, J., Adamek, L., Nizankowska, E., Szczeklik, A., Lam, B. K., Penrose, J. F., Austen, F. K., Holgate, S. T. and Sampson, A. P. (1998) Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. *J. Clin. Invest.* **101**, 834-846.
13. Lam, B. K. and Frank Austen, K. (2000) Leukotriene C4 synthase. A pivotal enzyme in the biosynthesis of the cysteinyl leukotrienes. *Am. J. Respir. Crit. Care. Med.* **161**, S16-19.
14. Celik, G., Bavbek, S., Misirligil, Z. and Melli, M. (2001) Release of cysteinyl leukotrienes with aspirin stimulation and the effect of prostaglandin E(2) on this release from peripheral blood leucocytes in aspirin-induced asthmatic patients. *Clin. Exp. Allergy* **31**, 1615-1622.
15. Szczeklik, A., Sanak, M., Nizankowska-Mogilnicka, E. and Kielbasa, B. (2004) Aspirin intolerance and the cyclooxygenase-leukotriene pathways. *Curr. Opin. Pulm. Med.* **10**, 51-56.
16. Vane, J. R., Bakhle, Y. S. and Botting, R. M. (1998) Cyclooxygenases 1 and 2. *Annu. Rev. Pharmacol. Toxicol.* **38**, 97-120.
17. Snijdewint, F. G., Kalinski, P., Wierenga, E. A., Bos, J. D. and Kapsenberg, M. L. (1993) Prostaglandin E2 differentially modulates cytokine secretion profiles of human T helper lymphocytes. *J. Immunol.* **150**, 5321-5329.
18. Betz, M. and Fox, B. S. (1991) Prostaglandin E2 inhibits production of Th1 lymphokines but not of Th2 lymphokines. *J. Immunol.* **146**, 108-113.
19. Coleman, R. A., Smith, W. L. and Narumiya, S. (1994) International Union of Pharmacology classification of prostanoid receptors: properties, distribution, and structure of the receptors and their subtypes. *Pharmacol. Rev.* **46**, 205-229.
20. Negishi, M., Sugimoto, Y. and Ichikawa, A. (1995) Molecular mechanisms of diverse actions of prostanoid receptors. *Biochim. Biophys. Acta.* **1259**, 109-119.
21. Narumiya, S., Sugimoto, Y. and Ushikubi, F. (1999) Prostanoid receptors: structures, properties, and functions. *Physiol. Rev.* **79**, 1193-1226.
22. Higashi, N., Taniguchi, M., Mita, H., Osame, M. and Akiyama, K. (2002) A comparative study of eicosanoid concentrations in sputum and urine in patients with aspirin-intolerant asthma. *Clin. Exp. Allergy* **32**, 1484-1490.
23. Kunikata, T., Yamane, H., Segi, E., Matsuoka, T., Sugimoto, Y., Tanaka, S., Tanaka, H., Nagai, H., Ichikawa, A. and Narumiya, S. (2005) Suppression of allergic inflammation by the prostaglandin E receptor subtype EP3. *Nat. Immunol.* **6**, 524-531.
24. Kim, S. H., Kim, Y. K., Park, H. W., Jee, Y. K., Kim, S. H., Bahn, J. W., Chang, Y. S., Kim, S. H., Ye, Y. M., Shin, E. S., Lee, J. E., Park, H. S. and Min, K. U. (2007) Association between polymorphisms in prostanoid receptor genes and aspirin-intolerant asthma. *Pharmacogenet. Genomics.* **17**, 295-304.
25. Jinnai, N., Sakagami, T., Sekigawa, T., Kakihara, M., Nakajima, T., Yoshida, K., Goto, S., Hasegawa, T., Koshino, T., Hasegawa, Y., Inoue, H., Suzuki, N., Sano, Y. and Inoue, I. (2004) Polymorphisms in the prostaglandin E2 receptor subtype 2 gene confer susceptibility to aspirin-intolerant asthma: a candidate gene approach. *Hum. Mol. Genet.* **13**, 3203-3217.
26. Park, H. W., Shin, E. S., Lee, J. E., Kim, S. H., Kim, S. S., Chang, Y. S., Kim, Y. K., Min, K. U., Kim, Y. Y. and Cho, S. H. (2007) Association between genetic variations in prostaglandin E2 receptor subtype EP3 gene (Ptger3) and asthma in the Korean population. *Clin. Exp. Allergy* **37**, 1609-1615.
27. Stock, J. L., Shinjo, K., Burkhardt, J., Roach, M., Taniguchi, K., Ishikawa, T., Kim, H. S., Flannery, P. J., Coffman, T. M., McNeish, J. D. and Audoly, L. P. (2001) The prostaglandin E2 EP1 receptor mediates pain perception and regulates blood pressure. *J. Clin. Invest.* **107**, 325-331.
28. Conklin, D., Jonassen, I., Aasland, R. and Taylor, W. R. (2002) Association of nucleotide patterns with gene function classes: application to human 3' untranslated sequences. *Bioinformatics* **18**, 182-189.
29. Kim, T. H., Chang, H. S., Park, S. M., Nam, B. Y., Park, J. S., Rhim, T., Park, H. S., Kim, M. K., Choi, I. S., Cho, S. H., Chung, I. Y., Park, B. L., Park, C. S. and Shin, H. D. (2008) Association of angiotensin I-converting enzyme gene polymorphisms with aspirin intolerance in asthmatics. *Clin. Exp. Allergy*. **38**, 1727-1737.
30. Cormican, L. J., Farooque, S., Altmann, D. R. and Lee, T. H. (2005) Improvements in an oral aspirin challenge protocol for the diagnosis of aspirin hypersensitivity. *Clin. Exp. Allergy.* **35**, 717-722.
31. Oliphant, A., Barker, D. L., Stuelpnagel, J. R. and Chee, M. S. (2002) BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. *Biotechniques* **32**, S56-61.
32. Livak, K. J. (1999) Allelic discrimination using fluorogenic probes and the 5' nuclease assay. *Genet. Anal.* **14**, 143-149.
33. Hedrick, P. W. (1987) Gametic disequilibrium measures: proceed with caution. *Genetics*. **117**, 331-341.
34. Stephens, M., Smith, N. J. and Donnelly, P. (2001) A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* **68**, 978-989.