ORIGINAL ARTICLE

Genetic variability of *prostaglandin E2* receptor subtype *EP4* gene in aspirin-intolerant chronic urticaria

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Prostaglandin E2 receptor subtype EP4 (PTGER4) is one of the four subtypes of receptors for prostaglandin E2 (PGE2). Overproduction of cysteinyl leukotriene in mast cells may be related with suppression of PGE2 in patients with aspirin hypersensitivity. Considering the association of PTGER4 in mast cells, urticaria- and aspirin-related disease, we hypothesized the genetic variability of *PTGER4* may be associated with aspirin-intolerant chronic urticaria (AICU). The case-control study was performed in 141 with AICU, 153 with aspirin-tolerant chronic urticaria (ATCU) and 174 with normal controls (NCs). *PTGER4* promoter single-nucleotide polymorphism was genotyped using a primer extension method with the SNAPshot ddNTP primer extension kit. The functional variability of *PTGER4* promoter polymorphism was carried out by dual-luciferase system and electrophoretic mobility shift assay (EMSA) in human mast cells (HMC-1). Furthermore, the effect of aspirin was performed for *PTGER4* mRNA expression using real-time PCR, and PGE2 production was checked in HMC-1 cells using ELISA. AICU patients carrying GG genotype at $-1254\text{ G} > \text{A}$ showed significantly higher frequency compared with NC ($P = 0.032$). Similarly, the minor allele frequency, G allele was significantly higher in AICU compared with NC ($P = 0.031$). *In vitro* functional study demonstrated that the -1254 G allele had lower luciferase activity ($P < 0.001$) in HMC-1 cells. EMSA finding showed that *PTGER4* -1254 G produced a specific band. Significantly decreased *PTGER4* expression ($P = 0.008$) and PGE2 production by aspirin exposure was confirmed in *in vitro* HMC cell line model ($P = 0.001$). The *PTGER4* -1254 G allele demonstrated a higher frequency in AICU patients and lower promoter activity with decreased expression of *PTGER4* and contributes to the development of AICU.

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INTRODUCTION

Aspirin ingestion can induce a wide range of clinically recognized allergic reactions, including aspirin-exacerbated respiratory disease (AERD), acetyl salicylic acid (ASA)-intolerant urticaria, chronic rhinitis and anaphylaxis.¹

Aspirin-intolerant chronic urticaria (AICU), a common type of ASA hypersensitivity presents aggravation of urticaria in patients suffering from chronic urticaria (CU), and its clinical symptoms are more severe compared to aspirin-tolerant CU.² Alteration in eicosanoid metabolism is the suggested pathogenesis for AICU.³

The prostaglandin E2 (PGE2), an arachidonic acid metabolite, is synthesized by diverse cell types in response to many stimuli and plays an important role in balancing Th1/Th2 immune responses.^{4,5} PGE2 has various effects such as pro-inflammatory and known to possess anti-inflammatory activities by inhibiting the release of inflammatory mediators from various cells.⁶ PGE2 binds to a specific group of

seven-transmembrane domain G-protein-coupled receptors of four subtypes, EP1, EP2, EP3 and EP4.⁷ Mast cells express EP2, EP3, EP4 receptors and respond to PGE2 and receptor-selective analogs with anticipated biochemical signatures and signaling events. PGE2 strongly suppresses allergic respiratory mucosal inflammation.⁸ PGE2 inhalation before allergen challenge prevents both early- and late-phase response in subjects with asthma⁶ and decreases the levels of prostaglandin D2 (PGD2) that are detected in the bronchoalveolar lavage fluid after allergen challenge.⁹ PGE2 inhibited immunoglobulin (Ig)E-mediated histamine and leukotriene C4 release from human lung mast cells.¹⁰ PGE2 inhalation prevents bronchoconstriction induced by challenge with nonselective COX inhibitors and abrogates the rise in urinary levels of LTE4, the end product of the cys-LTs.¹¹ These suggest that PGE2 inhibits mast cell activation in the respiratory tract. Overproduction of cys-LTs is unique to mast cells of AERD patients and is particularly sensitive to suppression by PGE2.¹²

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Therefore, an alternative hypothesis of ASA hypersensitivity regarding the blocking of synthesis of PGE2 by ASA due to COX inhibition is proposed to AERD and AICU.¹³

Prostaglandin receptor EP4 (PTGER4) is a prostaglandin receptor encoded by the *PTGER4* gene in humans and located in chromosome 5p13.1. This protein is one of four receptors identified for PGE2 receptor. Among these subtypes, EP2 and EP4 couple to G stimulatory (G_s), resulting in increases of intracellular cAMP concentrations,¹⁴ while EP3 couples to G inhibitory (G_i), causing a decrease in cAMP levels.¹⁵ *PTGER1*, *PTGER2*, *PTGER3* and *PTGER4* polymorphism are the important genetic factors affecting the risk and severity of asthma as well as pathogenesis of AERD.^{16–18} We recently reported that the other prostanoid receptor, thromboxane A2, (*TBXA2R*) –4684 T>C gene polymorphism is associated with aspirin-intolerant acute urticaria (AIAU). *TBXA2R* –4684 T allele may be associated with lower *TBXA2R* expression, which may contribute to the development of the AIAU phenotype.³

Most candidate gene approaches have focused on leukotriene-related pathways, whereas there have been no studies evaluating the effects of polymorphisms in PGE2 receptor genes on the development of AICU. Although *PTGER4* –1254A>G promoter polymorphism was previously studied in the case of AERD,¹⁶ no association study was noted in the case of AICU and functional study has not reported till now. Therefore, to replicate the effects of this promoter polymorphism in the other major phenotype of ASA hypersensitivity such as AICU, we performed genetic association study and to confirm the functional significance of promoter single-nucleotide polymorphism (SNP), we performed functional study in human mast cell 1 (HMC-1). The objective of this study was to determine the genetic contribution of SNPs in the *PTGER4* promoter region and its functional study to the phenotype of AICU.

MATERIALS AND METHODS

Study subjects

Two groups of CU patients, AICU ($n=141$) patients and aspirin-tolerant chronic urticaria (ATCU, $n=153$) based on the results of oral provocation up to 500 mg aspirin (Rhonal; KunWha Pharmaceutical, Seoul, Korea) were enrolled which were described previously.⁵ AICU patients who had AERD were excluded. Normal healthy controls (NC, $n=174$) with no personal or family history of allergic diseases or aspirin or drug hypersensitivity were recruited from the general Korean population. Informed consent was obtained from all subjects, and the institutional review board of Ajou University Hospital approved the study. Skin-prick tests were performed with 55 common aero-allergens (Bencard, West Sussex, UK), and atopy was defined as one or more positive reactions to common inhalant allergens. Total IgE concentrations were measured using the UniCAP system (Phadia, Uppsala, Sweden) according to the manufacturer's instructions.

DNA extraction, SNP identification and genotyping

Total genomic DNA was isolated from the peripheral blood of patients with AICU, ATCU and NC using a commercially available DNA isolation kit (Gentra Genomic DNA purification kit; Qiagen, Valencia, CA, USA) according to the manufacturer's protocol.

PTGER4 gene in promoter region in front of exon 1 was examined for single-nucleotide polymorphisms (SNPs) using the ABI Prism 3100 DNA analyzer (Applied Biosystems, Foster City, CA, USA). We previously identified four polymorphisms of –1598 T>C (rs45437592), –1529 T>C (rs413301), –1307 C>T (rs10039983) and –1254 A>G (rs45613037) in the promoter region, and found significant association of –1254 A>G with AERD.¹⁶ Therefore, in this study, we chose –1254 A>G SNP for genotyping with AICU, ATCU and NC using a primer extension method and the SNAPshot ddNTP primer extension kit. (Applied Biosystems, Foster City, CA, USA). The primer used for genotyping of –1254 A>G (rs45613037) SNP is forward primer,

5'-CTCCAACCTCTTTTATGGTG-3' and reverse primer, 5'-GGCTGTTCATC TTCTCTC-3' and genotyping primer, 5'-GCGTGAAAACACGGGAGCCG CCCC-3'.

Statistical analysis

The χ^2 -tests were used to detect a significant departure in genotype frequency from Hardy–Weinberg equilibrium at promoter SNP. Differences in the mean values of phenotypic characteristics among AICU patients were compared using χ^2 -test or Fisher's exact test and independent *t*-test. Continuous variables that did not have a normal distribution, such as the levels of serum total IgE, were log-transformed. Differences in the genotype frequency between the two groups were examined with the χ^2 -test and three logical regression models (co-dominant, dominant, recessive) were used after accounting for age and sex covariates. The dual-luciferase assay was assessed using an independent *t*-test. All statistical analyses were performed using SPSS version 12.0 (SPSS, Chicago, IL, USA). Statistical significance was established as $P \leq 0.05$.

Preparation of the PTGER4 construct for promoter assay

A 214-bp fragment of the human *PTGER4* gene was prepared by PCR amplification using either –1254 AA homozygous or –1254 GG homozygous human genomic DNA as a template (forward primer, 5'-GAGTTCACCT CCGCACCTC-3' and reverse primer, 5'-TCGCCTTCCACCCTCTGTAC-3'). Each PCR product was gel-purified with an agarose gel purification kit (iNtRON Biotechnology, Deajeon, Korea) and ligated into TA vector (Invitrogen, Deajeon, Korea). The plasmid was digested with *XhoI* and *HindIII* (Takara, Shuzo, Japan) and ligated into a similarly digested pGL3-basic luciferase reporter vector (Promega, Madison, WI, USA) using T4 DNA ligase (iNtRON Biotechnology). All constructs were verified by direct sequencing. Plasmid DNAs were prepared from these constructs using an Endo Free Plasmid Maxi kit (Qiagen, Hilden, Germany). Plasmid DNA concentrations and purity were assessed by UV spectrophotometry and agarose gel electrophoresis.

Transient transfection for luciferase activity

Transfection was performed in HMC-1 cells and repeated in U937 and Jurkat-T cells. Detailed transfection protocols were described in our previous study.¹⁹ HMC-1 was transfected using a microporator (model MP-100; Digital Bio Technology, Seoul, Korea) and PMK-1096 (Digital Bio Technology) according to the manufacturer's instructions. Briefly, 24-well plates were filled with 1000 μ l of Iscove's modified Dulbecco's medium containing 10% fetal bovine serum without antibiotics. Plates were incubated in a humidified incubator at 37°C under 5% CO₂. HMC-1 cells (2×10^5), reporter plasmid DNA containing *PTGER4* (1 μ g) and Renilla plasmid DNA (5 ng) were suspended in Solution R (10 μ l). The incorporation tube was filled with Solution E (3 ml). Cell and DNA mixtures were inserted into the microporator pipette station. HMC-1 cells were shocked using 1750 V/20 mA/plus width 1. The samples were transferred to preincubated 24-well plates and incubated for 24 h.

Cells were lysed with passive lysis buffer (Promega), and 100 μ l of lysate were added to each well. A 20- μ l aliquot from each well was assayed for luciferase activity using a dual-luciferase reporter assay kit (Promega) and Clarity Luminescence microplate reader (BioTek, Winooski, Vermont, USA). The pGL3-control (Promega) and the promoter-less pGL3-basic vectors were used as positive and negative controls, respectively. Transfection efficiency was determined by measuring Renilla activity using a dual-luciferase reporter assay kit (Promega) after co-transfection of the reporter construct and Renilla control vector.

Nuclear extract preparation and EMSA

HMC-1 cells were washed with ice-cold phosphate-buffered saline prior to suspension in ice-cold hypo osmotic buffer (10 mM HEPES, 2 mM MgCl₂, 0.1 mM EDTA, 10 mM KCl, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride, 1 mM NaVO₃, 10 mM NaF and 1 $\times$ protease inhibitors). Cells were incubated on ice for 10 min, 0.2% Nonidet P-40 was added, and the cells were incubated again for 15 min. After centrifugation at $15\,000 \times g$ for 30 s, the pellets were suspended in cold saline buffer containing 50 mM HEPES, 50 mM KCl, 300 mM NaCl, 0.1 mM EDTA, 10% glycerol, 1 mM dithiothreitol, 1 mM

phenylmethylsulfonyl fluoride, 1 mM NaVO₃, 10 mM NaF and 1 × protease inhibitor. The mixture was incubated on ice for 30 min. After centrifugation at 15 000 × g for 30 min at 4 °C, the supernatants containing the nuclear proteins were collected. The protein concentrations were determined by Bradford assay. Nuclear protein aliquots (5 µg) were stored at –80 °C until use. Double-stranded oligonucleotides for *PTGER4* and the specific competitor SP-1 were used as probes for electrophoretic mobility shift assay. The double-stranded oligonucleotides were annealed at 95 °C for 5 min and gradually cooled at room temperature. The double-stranded DNA probes were radiolabeled at their 5'-ends using DNA polymerase I (Klenow; Promega) and [α -³²P]-CTP. A 10-µl reaction mixture comprising nuclear protein (5 µg), 75.2 mM NaCl, 20 mM HEPES, 1 mM EDTA, 5% glycerol, 0.0504 mM MgCl₂, 5 mM dithiothreitol, 1 mM NaVO₃, 10 mM NaF, 400 ng of bovine serum albumin, and 250 ng of poly (dI-dC) was preincubated on ice for 20 min. Radiolabeled probe (0.2 pmol) was added, and the reaction was incubated on ice for another 30 min. The reaction mixture was then subjected to electrophoresis in a native 6% polyacrylamide gel. A competition experiment was performed using unlabeled, blunt-ended competitor oligonucleotides added to the binding reaction prior to the addition of radiolabeled oligonucleotide. Gels were dried, and radioactivity was detected using a FLA3000 scanner (Fuji Photo Film, Tokyo, Japan).

ASA decreased the *PTGER4* mRNA expression in HMC-1 cell line
HMC-1 cell line (5×10^5) was stimulated with ASA at 2 mM of dose for 24 h and cells were harvested and total RNA was extracted from cells using easy-blue reagent (iNtRON Biotechnology) according to the manufacturer's instruction. cDNA was synthesized, confirmed and real-time PCR was performed to check the mRNA expression of *PTGER4* using *PTGER4*-specific primers 5'-tgaa-cagccagtgaccatc-3' as a forward primer and 5'-accaacaagtgccaacag-3' as a reverse primer. Normalization was performed using β -actin primer 5'-tcct tctgcatcctgtcggc-3' as a forward primer and 5'-caagagatggccacggctgc-3' as a reverse primer.

ASA decreased the PGE₂ production from HMC-1 cell line
HMC-1 cell line (5×10^5) was stimulated with phorbol-12-myristate 13-acetate 10 ng µl⁻¹ and 10 µM Ca²⁺ for 30 min using IMDM serum-free media. Cells were washed with phosphate-buffered saline and aspirin 2 mM was treated

for 24 h. Supernatant was collected and measured PGE₂ production using prostaglandin E₂ ELISA kit (Cayman, 514010, Ann Arbor, MI, USA).

RESULTS

Clinical characteristics of the study subjects

The clinical characteristics of the study population are summarized in Table 1. The atopy rate and total serum IgE (log-transformed) level were significantly higher in AICU groups compared with the ATCU and NC groups ($P < 0.001$, $P < 0.001$), respectively.

Genotype and allele frequencies of the *PTGER4* gene

The genotype distribution of the promoter polymorphism –1254A>G of *PTGER4* did not differ significantly from Hardy–Weinberg equilibrium (data were not shown, $P > 0.05$), which was shown by χ^2 -test. The genotype frequency of *PTGER4* –1254A>G differed significantly between the AICU and NC groups in the co-dominant model (Table 2); however, no significant difference was noted between the AICU and ATCU groups. AICU patients demonstrated a significantly higher frequency of the *PTGER4* –1254A>G homozygous GG genotype compared with NC subjects, and the AA genotypes occurred significantly less frequently in AICU patients than in NC subjects ($P = 0.032$).

Effect of the *PTGER4* –1254A>G polymorphism on promoter activity

To assess whether the *PTGER4* –1254A>G polymorphism is associated with altered promoter activity, constructs containing either *PTGER4* –1254A or *PTGER4* –1254G and a luciferase reporter gene were transfected into HMC-1 cells. Luciferase activity was significantly lower with the *PTGER4* –1254G construct than with the –*PTGER4* –1254A construct ($P < 0.001$; Figure 1b). Further transfection experiments performed in Jurkat-T cell and U937 cells yield similar results: significantly lower promoter activity with the *PTGER4* –1254G

Table 1 Clinical characteristics of the study subject

	AICU (N = 141)	ATCU (N = 153)	NC (N = 174)	P-value		
				AICU vs ATCU	AICU vs NC	ATCU vs NC
Sex (male/total)	69/141 (48.9%)	73/153 (47.7%)	77/174 (44.3%)	0.907	0.428	0.579
Age (years)	35.44 ± 11.54/141	39.39 ± 11.71/153	30.97 ± 10.68/173	0.004	<0.001	<0.001
Atopy (presence/total)	101/140 (72.1%)	75/152 (49.3%)	16/142 (11.3%)	<0.001	<0.001	<0.001
Log(serum total IgE (IU ml ⁻¹))	5.20 ± 1.05/121	4.69 ± 1.11/144	3.57 ± 1.30/44	<0.001	<0.001	<0.001

Abbreviations: AICU, aspirin-intolerant chronic urticaria; ATCU, aspirin-tolerant chronic urticaria; NC, normal control.
P-values less than 0.05 were indicated in bold.

Table 2 The genotype and allele frequencies of *PTGER4* gene polymorphism

Loci	Genotype	AICU (N = 141)	ATCU (N = 153)	NC (N = 174)	P-value		
					AICU vs ATCU	AICU vs NC	ATCU vs NC
–1254A>G	AA	76 (53.9%)	85 (55.6%)	107 (61.5%)	^a 0.86	^a 0.032	^a 0.108
	AG	54 (38.3%)	59 (38.6%)	59 (33.9%)	^b 0.697	^b 0.075	^b 0.239
	GG	11 (7.8%)	9 (5.9%)	8 (4.6%)	^c 0.982	^c 0.075	^c 0.16
	q	0.27	0.252	0.216	0.861	0.031	0.112

Abbreviations: AICU, aspirin-intolerant chronic urticaria; ATCU, aspirin-tolerant chronic urticaria; NC, normal controls.
Each P-value was calculated with ^aco-dominant (AA vs GG), ^bdominant (AA + AG vs GG) and ^crecessive model (AA vs AG + GG), respectively.
The fourth P-value was calculated according to minor allele frequency, q. logistic regression analysis was applied to control for age and sex as covariates in all models.
P-values less than 0.05 were indicated in bold.

Figure 1 Effect of $-1254A>G$ polymorphism on the transcription activity of *PTGER4* promoter. (a) Luciferase reporter constructs using transfected (b) HMC-1, (c) U937 and (d) Jurkat-T cell. Relative luciferase activity was represented as the ratio of the activity to the Luciferase activity in the cells transfected with the empty control vector pGL3 basic. Luciferase activity assay was performed in three independent experiments ($n=9$).

construct compared with the *PTGER4* $-1254A$ construct in Jurkat-T cells ($P=0.003$; Figure 1c) and U937 cells ($P<0.001$; Figure 1d). To identify possible transcription factor-binding sites in this promoter region, transcription factor databases were searched using the CONSITE program (<http://asp.ii.uib.no:8090/cgi-bin/CONSITE/consite>). As the *PTGER4* $-1254A>G$ polymorphism creates a potential SP-1 transcription factor-binding site, we chose SP-1 as a candidate transcription factor for competition assay to examine the effect of the *PTGER4* polymorphism.

Effects of *PTGER4* $-1254A>G$ gene polymorphism on transcriptional activity

In an electrophoretic mobility shift assay of nuclear extracts from HMC-1 cells using a double-stranded oligonucleotide probes corresponding to *PTGER4* $-1254A>G$, $-1254G$ produced a specific band with higher affinity than that produced by $-1254A$. The band disappeared in the presence of non-labeled $-1254G$ probe as a competitor, but not in the presence of non-labeled $-1254A$ probe (Figure 2) or in the presence of the transcription factor SP-1 or nuclear factor- κ B.

Effect of ASA on *PTGER4* mRNA expression and PGE2 production from HMC-1 cells

PTGER4 mRNA expression was significantly decreased after ASA treatment in HMC-1 cell line at 2 mM ($P=0.008$) as shown by real-time PCR (Figure 3). PGE2 level (pg/ml) was significantly decreased ($P=0.001$) after ASA treatment, as measured by supernatant from HMC-1 cells, as shown in Figure 4.

DISCUSSION

Change in eicosanoid metabolism is widely accepted mechanism for ASA hypersensitivity; however, the detail mechanism for AICU is yet to be known. To our knowledge, this is the first study to investigate a significant association between AICU and promoter polymorphisms of *PTGER4*. The *PTGER4* $-1254A>G$ promoter polymorphism was shown to be significantly associated with the AICU group, which was further supported by several steps of comparable functional studies.

a

PTGER4_1254A:GGAGCCGCCCCACCCCGCGCGCC
PTGER4_1254G:GGAGCCGCCCCGCCCCGCGCGCC
SP1:ATTCGATCGGGGCGGGGCGA
NF- κ B:AGTTGAGGGGACTTCCCAGG

Figure 2 Results of an electrophoretic mobility shift assay for the *PTGER4* $-1254A>G$ polymorphism using nuclear extracts obtained from (a) nucleotide sequences of oligonucleotides used as probes and competitors ($-1254T$, $-1254C$, SP-1, nuclear factor- κ B). (b) Differential binding of nuclear protein to $-1254G/A$ alleles in HMC-1 cells. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

In this study, our result indicate that $-1254GG$ is the risk-associated genotype of the *PTGER4* $-1254A>G$ promoter polymorphism in an association with AICU. It was reported in the Korean population that the polymorphisms in the *PTGER1*, *PTGER2*, *PTGER3* and *PTGER4* gene are known to be associated with AERD.⁷ Moreover, it was reported that the frequencies of GG homozygotes or heterozygotes of $-1254A>G$ were significantly higher in AERD

Figure 3 Expressions of PTGER4 from real-time PCR. Significant difference of mRNA expression of PTGER4 is found between aspirin-untreated and -treated human mast cell (HMC-1; $n = 3 \times 3 = 9$, $P = 0.008$).

Figure 4 Effect of aspirin in PGE2 production from HMC-1 cell line. Significant difference was found in ASA-treated and -untreated sample in HMC-1 cells ($n = 3 \times 3 = 9$, $P = 0.001$).

patients than in ATA patients or NCs.¹⁶ This result indicates that the importance of genetic variations in *PTGER4* –1254A>G to ones predisposition to AICU is similar with AERD.

Mast cells are the key cell model for urticaria and release histamine and other chemical mediators, which produce urticarial symptoms,²⁰ and mast cell activation has been reported in ASA-intolerant urticaria patients.²¹ Besides, PGE2 could suppress excessive anti-IgE-induced cysteinyl leukotriene production in mast cells in patients with AERD.¹² Our result confirmed that the functional study of *PTGER4* promoter polymorphism in HMC-1 cell line showed differential transcriptional regulation by dual-luciferase activity. The decreased promoter activity observed for *PTGER4* –1254G compared with –1254A suggests downregulation of PTGER4 expression on inflammatory cells in the presence of PTGER4 G allele. Thus, ASA exposure may cause lower expression of PTGER4 with decreased

protective effect of PGE2 in subjects with this –1254G allele, leading to develop AICU.

In addition, the electrophoretic mobility shift assay result demonstrated that –1254G-specific DNA binding was more pronounced and might have been important in the transcriptional regulation of the *PTGER4* gene, compared with –1254A. It was further confirmed by competition assay, which was performed using –1254G probe. The transcription factor database suggested SP-1 as a candidate transcription factor; however, SP-1 did not show any competition with the –1254G-specific band. This indicates that –1254G allele may bind with other regulatory factor for transcriptional regulation of *PTGER4* promoter polymorphism; however, it needs further study to clarify specific pathway of transcriptional regulation.

On the basis of the expression of PTGER4 receptors on mast cells,⁸ we performed additional *in vitro* functional study to confirm the effect of ASA on PTGER4 expression and PGE2 level in human mast cells. Our result showed lower mRNA expression of PTGER4 by ASA exposure with decreased production of PGE2 from HMC-1 cells. Therefore, lower promoter activity by –1254G allele may be responsible for lower production of PGE2, the protective prostanoid in the airway inflammation and decreased the protective effect on AICU after ASA exposure. Alternatively, it could enhance other pro-inflammatory cytokines and cysteinyl leukotrienes from inflammatory cells.²²

It was previously noted that the suppression of tumor necrosis factor- α protein and mRNA expression by PGE2⁸ while EP4 agonist significantly reduced levels of proinflammatory cytokines and chemokines, indicating the role of EP4 activation in systemic inflammation.²³

Despite the genetic and functional study data provided here, replication studies in larger and different cohorts must be in consideration to validate these findings.

In conclusion, these findings suggest that –1254A>G polymorphism of the *PTGER4* gene is associated with the AICU phenotype. The *PTGER4* –1254G allele with a higher frequency in AICU patients demonstrated lower promoter activity and expression of PTGER4, which will contribute to the development of AICU.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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