Impaired E Prostanoid₂ Expression and Resistance to Prostaglandin E₂ in Nasal Polyp Fibroblasts from Subjects with Aspirin-Exacerbated Respiratory Disease

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Abstract

Recurrent, rapidly growing nasal polyps are hallmarks of aspirin-exacerbated respiratory disease (AERD), although the mechanisms of polyp growth have not been identified. Fibroblasts are intimately involved in tissue remodeling, and the growth of fibroblasts is suppressed by prostaglandin E₂ (PGE₂), which elicits antiproliferative effects mediated through the E prostanoid (EP)₂ receptor. We now report that cultured fibroblasts from the nasal polyps of subjects with AERD resist this antiproliferative effect. Fibroblasts from polyps of subjects with AERD resisted the antiproliferative actions of PGE₂ and a selective EP₂ agonist ($P < 0.0001$ at 1 μ M) compared with nasal fibroblasts from aspirin-tolerant control subjects undergoing polypectomy or from healthy control subjects undergoing concha bullosa resections. Cell surface expression of the EP₂ receptor protein was lower in fibroblasts from subjects with AERD than in fibroblasts from

healthy control subjects and aspirin-tolerant subjects ($P < 0.01$ for both). Treatment of the fibroblasts with trichostatin A, a histone deacetylase inhibitor, significantly increased EP₂ receptor mRNA in fibroblasts from AERD and aspirin-tolerant subjects but had no effect on cyclooxygenase-2, EP₄, and microsomal PGE synthase 1 (mPGES-1) mRNA levels. Histone acetylation (H3K27ac) at the EP₂ promoter correlated strongly with baseline EP₂ mRNA ($r = 0.80$; $P < 0.01$). These studies suggest that the EP₂ promoter is under epigenetic control, and one explanation for PGE₂ resistance in AERD is an epigenetically mediated reduction of EP₂ receptor expression, which could contribute to the refractory nasal polyposis typically observed in this syndrome.

Keywords: aspirin-exacerbated respiratory disease; prostaglandin E₂; E prostanoid type 2 receptor; histone acetylation; DNA methylation

Aspirin-exacerbated respiratory disease (AERD) is an acquired disorder characterized by stereotypical upper and lower respiratory reactions to aspirin and other cyclooxygenase-1 (COX)-1 inhibitors. Symptom onset most often occurs after

puberty through age 40 without a clear genetic predisposition or a known environmental or infectious trigger. Aggressive nasal polyposis is a hallmark of AERD and is characterized by high polyp burden, abundance of eosinophils and mast

cells in the polyp tissue, extracellular matrix deposition, and regrowth of polyps as early as several weeks after polypectomy (1). The rapid regrowth of polyps after surgical excision leads to persistent anosmia, impaired sleep, chronic sinusitis, and

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Clinical Relevance

This work demonstrates that impairment of E prostanoid (EP)₂ protein expression in nasal polyp fibroblasts from patients with aspirin-exacerbated respiratory disease (AERD) results in resistance to the antiproliferative effects of prostaglandin E₂ and EP₂ signaling and provides an epigenetic basis for EP₂ expression. Here we provide the first evidence that epigenetic modifications may contribute to the development of AERD.

worsening of lower respiratory disease. Aspirin desensitization followed by high-dose daily aspirin therapy, which is clinically effective in 60 to 80% of patients with AERD who can tolerate therapy at 1 year, is the only available therapeutic option demonstrated to retard nasal polyp growth (2, 3). The mechanism of aggressive polyp regrowth in AERD is unknown, but it is likely to involve a fundamental disturbance in endogenous regulation of cellular proliferation and tissue remodeling.

The pathogenesis of AERD involves both dysregulation of arachidonic acid (AA) metabolism and aberrant function of receptors for AA metabolites. Prostaglandin (PG) E₂ is generated from AA by the COX isoenzymes COX-1 and COX-2, which generate the precursor PGH₂. Three terminal PGE₂ synthases (PGES), termed cytosolic PGES and microsomal PGES (mPGES)-1 and mPGES-2, isomerize PGH₂ to PGE₂. COX-1 is constitutively active, whereas COX-2 is inducible by proinflammatory stimuli, including IL-1 β (4). Up-regulation of COX-2 expression is accompanied by parallel up-regulation of mPGES-1, ensuring that PGE₂ generation is enhanced with inflammation. PGE₂ signals through four different E prostanoid (EP) receptors. The antifibrotic functions of PGE₂ in respiratory tissue largely reflect its actions at the EP type 2 (EP₂) receptor (5). Like mPGES-1, EP₂ receptor expression is up-regulated in tandem with COX-2 in several cell types (6). EP₂ receptor stimulation suppresses the growth and activation of fibroblasts, the major effector cells of tissue remodeling, which are also abundant sources of PGE₂. Fibroblasts from

nasal polyps of patients with AERD show less inducible expression of COX-2, generate significantly less PGE₂ than do nasal polyp fibroblasts from aspirin-tolerant (AT) control subjects, and express less EP₂ receptor protein (6). Although altered PGE₂ synthesis and/or EP₂ receptor expression by fibroblasts could potentially contribute to the rapid growth of nasal polyps in AERD, neither the functional consequences of these perturbations nor their mechanistic basis are known.

Although candidate gene association studies have searched for a genetic basis for AERD, the lack of substantial familial clustering of AERD and its onset in adulthood argues for an acquired molecular defect, possibly of epigenetic origin. Epigenetic modifications, such as acetylation/deacetylation of histones and methylation of CpG islands in promoters, can significantly alter gene expression. The PGE₂ synthetic pathway and receptor signaling systems are potential targets of such modifications. In idiopathic pulmonary fibrosis, reduced histone acetylation of the COX-2 promoter region impairs expression of the COX-2 enzyme in lung fibroblasts, and hypermethylation of the EP₂ (PTGER2) promoter region has been associated with decreased EP₂ protein expression in fibroblasts (7, 8). Assessment of global methylation patterns in nasal polyp tissue from subjects with AERD identified hypermethylation of the PTGES gene encoding mPGES-1 when compared with nasal polyp tissue from AT subjects with asthma (9). Whether alterations in DNA methylation levels or histone acetylation/deacetylation balance result in defects in EP₂ expression in AERD is not known, but such epigenetic changes seem plausible given the adult onset, persistent nature, and lack of heritability of the disease.

Because of the known effects of PGE₂ on fibroblast growth, we hypothesized that an intrinsic defect in EP₂ expression of nasal polyp fibroblasts allows for their unchecked proliferation in AERD and may underlie the aggressive nasal polyp growth that plagues these patients. We sought to confirm the impairment of EP₂ protein expression in nasal polyp fibroblasts from patients with AERD, to evaluate the functional consequences, and to examine its potential epigenetic basis. Given the antifibrotic properties of PGE₂, we

hypothesized that dysregulated EP receptor function on fibroblasts could contribute to the pathogenesis of nasal polyps. Some of the results of these studies have been previously reported in the form of an abstract (10).

Materials and Methods

Subject Selection, Tissue Harvesting, and Cell Culture

Patients were recruited from the Allergy, Pulmonary, and Otolaryngology clinics at the Brigham and Women's Hospital (Boston, MA) and the Allergy and Otolaryngology clinics at the University of Virginia Health System (Charlottesville, VA). Healthy control subjects had no history of asthma, intolerance to COX-1 inhibitors, chronic rhinosinusitis, or nasal polyposis and were undergoing sinus surgery for concha bullosa. AT control subjects had chronic rhinosinusitis with polyposis, had no history of asthma, and had taken a nonselective COX-1 inhibitor within the previous 6 months without adverse reaction. All patients with AERD had asthma, nasal polyposis, and a history of respiratory reaction on ingestion of a nonselective COX-1 inhibitor confirmed with an oral challenge to aspirin. None of the subjects smoked. The polyp or concha bullosa tissue excised at surgery was mechanically dissected, isolated by a previously described protocol (11), and maintained in RPMI with 10% FBS and 1% penicillin-streptomycin at 37°C with 99% relative humidity and 5% CO₂. All assays were performed between passages 4 through 10. Institutional Review Boards approved the study, and all subjects provided written consent.

Proliferation Assays

Fibroblasts were stimulated with forskolin, PGE₂, or the EP₂ receptor-specific agonist ONO-AE1-259-01 for 24 hours. Tritiated thymidine (³H-TdR; 1 μ Ci) was added to each well. Radioactivity in counts per minute was assessed using a beta-counter 24 hours later.

Flow Cytometry

Fibroblasts were stained with a mouse anti-human monoclonal antibody directed against the extracellular portions of the human EP₂ receptor (12), anti-human

Table 1. Clinical Characteristics of Subjects

Demographics	Healthy Control	Aspirin-Tolerant	AERD
<i>n</i>	8	9	18
Male, %	60%	33%	57%
Age at surgery, yr (SD)	42.8 (20.6)	39.7 (13.6)	47 (11)

Definition of abbreviation: AERD, aspirin-exacerbated respiratory disease.

CD90, anti-human CD45, or an IgG1 isotype control.

Quantitative PCR

Fibroblasts were stimulated for 24 hours with IL-1 β or trichostatin A (TSA). RNA was extracted using the RNeasy Plus Mini Kit (Qiagen, Germantown, MD). Relative quantities of mRNA were determined by comparison with GAPDH.

Histone Acetylation

Formaldehyde-fixed chromatin was precipitated by phenol/chloroform extraction. Immunoprecipitation was

performed using Dynabeads prepared with anti-H3K27Ac antibodies, followed by reverse crosslinking and DNA purification using the Qiagen MinElute Kit. Quantitative PCR was performed targeting known H3K27ac binding sites at the PTGER2 and PTGER4 promoters (see Table E1 in the online supplement), as well as the GAPDH promoter (positive control) and a gene desert (negative control). Percent acetylation was calculated based on the ratio between the acetylation at EP₂ and the delta between GAPDH and gene desert for each sample.

DNA Methylation

One microgram of extracted genomic DNA was treated with sodium bisulfite using the EZ DNA Methylation Kit (Zymo Research, Orange, CA). DNA methylation analyses on bisulfite-treated DNA were performed using highly quantitative analysis based on bisulfite-pyrosequencing (13) (see Table E2 for sequence details). Six CpGs were evaluated in the assay.

Statistical Analysis

The data are presented as the mean $\pm$ SEM unless otherwise stated. Differences in values were assessed using one-way ANOVA with *post hoc* analysis by a two-tailed *t* test for nonpaired samples for all data unless otherwise stated. Significance was defined as $P < 0.05$. Effect size was measured with Spearman's rank correlation coefficient.

Further details of the methods are included in the online supplement.

Results

Patient Characteristics

Thirty-three subjects were enrolled for this study, including 18 patients with AERD, nine AT control subjects, and eight healthy control subjects. The three groups of patients did not differ statistically in sex or age at the time of surgery (Table 1).

Isolation of Fibroblasts Surgically Excised Nasal Tissue Results in Pure Culture Populations by Passage 4

To ensure our fibroblast isolation and culture protocol yielded a pure fibroblast population, we assessed the presence of CD90 (Thy-1) and CD45 on fibroblasts from all three groups. All cells in all groups showed uniform expression of CD90 and lacked CD45 (a representative histogram is shown in Figure E1 in the online supplement).

Fibroblasts from Subjects with AERD Proliferate Rapidly and Exhibit Relative Resistance to the Antiproliferative Effects of PGE₂

We measured ³H-TdR incorporation to determine whether cultured nasal polyp fibroblasts from patients with AERD exhibited differential growth rates and responses to PGE₂ as compared with AT and control subjects. Fibroblasts from sinonasal tissue of healthy subjects with AT

Figure 1. Fibroblast proliferation. Fibroblasts from nasal polyps of subjects with aspirin-exacerbated respiratory disease (AERD) ($n = 11$ – 12), aspirin-tolerant (AT) sinus disease ($n = 6$), or healthy control subjects ($n = 5$) were plated overnight and then serum-starved and treated with no stimulant, forskolin 100 μ M, prostaglandin E₂ (PGE₂) (10 μ M), or an E prostanoid 2 (EP₂) agonist (ONO-AE1-259, 0.01–1 μ M) along with tritiated thymidine (³H-TdR) for 24 hours, and the incorporated ³H-TdR was recorded in counts per minute (cpm). Intrinsic baseline rate of proliferation (A) and percent inhibition of proliferation in response to forskolin, PGE₂, and the selective EP₂ agonist (B) are shown. Data are expressed as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, and **** $P < 0.0001$.

Figure 2. EP₂ receptor cell surface and mRNA expression on fibroblasts from healthy and AT control subjects compared with those from subjects with AERD. Cultured fibroblasts were serum starved for 48 hours and stained with EP₂ monoclonal antibodies. (A) A representative histogram of EP₂ cell surface expression of fibroblasts from a healthy control subject, an AT subject, and a patient with AERD are shown, along with the appropriate isotype control. (B) The percent of fibroblasts that

and subjects with AERD were treated with forskolin (100 nM), PGE₂ 10 (μM), or a selective EP₂ agonist (0.01–1 μM, ONO-AE1-259), followed by assessment of proliferation rates by measuring the incorporation of ³H-TdR over 24 hours. Fibroblasts from nasal polyp tissue of patients with AT disease and AERD demonstrated higher baseline proliferation rates than fibroblasts from healthy control nasal mucosa ($P < 0.01$ and $P < 0.05$, respectively) (Figure 1A). Treatment of the cells with indomethacin to suppress endogenous PGE₂ production did not alter the growth rates (data not shown). However, fibroblasts derived from patients with AERD demonstrated significantly impaired responsiveness to both PGE₂ and the EP₂ receptor agonist. Whereas PGE₂ suppressed fibroblast proliferation in AT patients ($66.1 \pm 7.0\%$) (Figure 1B), AERD fibroblasts were significantly less responsive to PGE₂ ($12.8 \pm 8.8\%$ suppression; $P < 0.01$). This defect became more apparent after treatment with the selective EP₂ receptor agonist: fibroblast proliferation was significantly suppressed in samples from healthy control subjects ($42.2 \pm 4.9\%$) and AT patients ($39.5 \pm 8.3\%$, at a suppression plateau dose of 0.1 μM) as compared with samples from patients with AERD ($10.9 \pm 3.6\%$ at 0.1 μM, $P < 0.01$ and $P < 0.001$; $9.3 \pm 3.8\%$ at 1 μM, $P < 0.0001$ and $P < 0.0001$, respectively) (Figure 1B). Forskolin, used as a stimulus to activate cAMP accumulation independently of EP receptors, suppressed proliferation of fibroblasts from all groups, particularly those from AT and AERD nasal polyps. The function and signaling of the fibroblasts remained stable up to passage 10 (Figure E2).

Fibroblasts from AERD Nasal Polyps Show Diminished EP₂ Receptor Expression

To determine whether EP₂ receptor protein and mRNA expression were lower on nasal

polyp fibroblasts from subjects with AERD than on healthy and AT fibroblasts, we performed cytofluorographic and quantitative PCR analyses. Figure 2A shows a representative histogram of cell surface EP₂ protein expression. Unstimulated fibroblasts from patients with AERD had significantly lower levels of cell surface EP₂ receptor expression ($3.8 \pm 1.0\%$ of cells not overlapping with isotype control; net mean fluorescence intensity, 29.9 ± 5.6) than did fibroblasts from control subjects with AT ($29.4 \pm 13.5\%$; net mean fluorescence intensity, 86.8 ± 32.3 ; $P < 0.05$) (Figures 2B and 2C). Baseline expression of EP₂ receptor mRNA was very low and was similar between the groups (Figure 2D).

EP₂ Receptor Expression Is Inducible by Inhibition of Histone Deacetylation

To determine whether EP₂ receptor could be up-regulated in fibroblasts from subjects with AERD and whether this paralleled changes in the other PGE₂ pathway genes, we stimulated fibroblasts from AERD ($n = 9-13$), AT ($n = 3$), and healthy control nasal mucosa ($n = 5$) with IL-1β (1 ng/ml) or with the histone deacetylase inhibitor TSA (5 μM). We collected RNA after 24 hours. IL-1β up-regulated the expression of COX-2 and mPGES-1 mRNAs in AERD ($P < 0.001$ and $P < 0.001$, respectively), AT ($P < 0.01$ and $P < 0.05$, respectively), and healthy control ($P < 0.01$ and $P < 0.01$, respectively) fibroblasts but did not significantly up-regulate the expression of EP₂ or EP₄ mRNA in any group (Figure 3A). TSA significantly increased EP₂ mRNA expression from baseline in fibroblasts from subjects with AERD (8.3 ± 2.8 -fold increase; $P < 0.01$) and AT subjects (19.88 ± 5.7 -fold increase; $P < 0.01$), with a similar trend in the control fibroblasts (4.6 ± 2.3 -fold increase; $P = 0.06$). The magnitude of TSA induction on EP₂ mRNA trended to be greater in the AERD and AT groups but was not statistically different from the healthy control subjects (ANOVA; $P = 0.09$). TSA

Figure 2. (Continued). expressed surface EP₂ (percent of EP₂-positive fibroblasts minus percent of isotype control positive fibroblasts) is shown from healthy control subjects ($n = 5$), AT control subjects ($n = 3$), and subjects with AERD ($n = 7$). (C) Mean fluorescence intensity (MFI) of EP₂ antibody minus MFI of isotype control of fibroblasts from healthy control subjects ($n = 5$), AT control subjects ($n = 3$), and subjects with AERD ($n = 7$). (D) Baseline EP₂ mRNA expression after serum starvation for 24 hours in healthy fibroblasts ($n = 5$), AT fibroblasts ($n = 3$), and AERD fibroblasts ($n = 11$). Data are expressed as individual subjects with group mean $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$. GAPDH, glyceraldehyde 3-phosphate dehydrogenase.

treatment did not alter the levels of COX-2, mPGES1, or EP₄ mRNA (Figure 3B).

Histone Acetylation at *PTGER2* Correlates with Baseline EP₂ Receptor mRNA Expression on Nasal Fibroblasts

Cultured fibroblasts from healthy control subjects ($n = 3$) and subjects with AERD ($n = 6$) were serum starved with or without IL-1 β (1 ng/ml) for 24 hours.

Formaldehyde fixation followed by chromatin immunoprecipitation (XChIP) was performed, and enrichment of histone H3 lysine 27 acetylation (H3K27ac), a histone marker for an active enhancer or promoter (13), was assessed at the *PTGER2* and *PTGER4* promoters (Table E1). There was variable baseline histone acetylation at *PTGER2* (Figure 4A). Baseline EP₂ mRNA correlated ($r = 0.80$; $P < 0.01$) with enrichment of H3K27ac in the promoter of *PTGER2* in nasal polyp fibroblasts from all subjects (Figure 4B). There was no relationship between EP₄ mRNA and H3K27ac at *PTGER4* (data not shown). Treatment with IL-1 β had no effect on H3K27 acetylation at *PTGER2* (data not shown).

Methylation at *PTGER2* and *PTGS2* Is Not Different between Groups

Cultured fibroblasts serum starved with or without IL-1 β (1 ng/ml) for 24 hours were subjected to bisulfate modification followed by pyrosequencing to assess DNA methylation. DNA methylation at the EP₂ promoter in fibroblasts from subjects with AERD (49.5 ± 8.1 ; $n = 3$), AT subjects (38.3 ± 12.6 ; $n = 3$), and healthy control subjects (52.4 ± 7.6 ; $n = 3$) was not different (data not shown). DNA methylation assessed at six positions of the *PTGS2* promoter ranged from 0.7 to 4.3% methylation ($2.1 \pm 0.7\%$ [$n = 3$] for control subjects and subjects with AERD; 6.8 ± 4.8 [$n = 3$] for control subjects with AT) and showed no difference between all three groups of fibroblasts. Treatment with IL-1 β (1 ng/ml) had no effect on methylation in any group at *PTGER2* or *PTGS2* (data not shown).

Discussion

Defects in PGE₂ generation, EP₂ receptor expression, and downstream signaling systems have been described in multiple cell

Figure 3. (A) To study the effects of IL-1 β on mRNA expression of EP₂, EP₄, cyclooxygenase (COX)-2, and microsomal PGE synthase 1 (mPGES-1), cultured fibroblasts were serum starved and incubated in the presence or absence of IL-1 β (1 ng/ml) for 24 hours (healthy, $n = 4$; AT, $n = 3$; AERD $n = 11$ –13). Ratio paired t test was used to assess change from baseline. Data are expressed as fold change from baseline for individual subjects with group mean. (B) To study the effects of trichostatin A (TSA) on PGE₂ pathway gene expression, cultured fibroblasts from healthy control subjects ($n = 5$), AT subjects ($n = 3$), and subjects with AERD ($n = 9$ –13) were serum starved and incubated in the presence of TSA or left untreated for 24 hours. EP₂, COX-2, mPGES-1, and EP₄ mRNA levels were determined by quantitative PCR with GAPDH used as a control and compared with untreated fibroblasts. Ratio paired t test was used to assess change from baseline. Data are expressed as fold change over baseline for individual subjects with group mean. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 4. Histone acetylation at *PTGER2* in cultured nasal fibroblasts from AT control subjects and subjects with AERD. (A) Histone acetylation levels at *PTGER2* promoter are shown for nine subjects. (B) Correlation between baseline EP₂ mRNA expression and baseline H3K27ac at *PTGER2* in fibroblasts from all samples (healthy, $n = 3$; AERD, $n = 6$) was determined by Spearman's correlation coefficient ($r = 0.80$; $P < 0.01$).

types in AERD (6, 12, 14–16). PGE₂ is a potent inhibitor of fibroblast proliferation primarily by stimulating cyclic AMP accumulation and exchange protein activated by cyclic AMP (EPAC) through EP₂ receptors (17). Fibroblasts and inflammatory cells from nasal polyps of subjects with AERD show reduced EP₂ receptor protein compared with AT control subjects (6, 15). Although such reductions could have substantial pathophysiologic consequences, no previous studies had addressed the functionality of EP₂ receptors on fibroblasts from the pathologic tissue in AERD, and no mechanism had been inferred for reduced expression.

In our initial experiments, we found that nasal polyp fibroblasts from subjects with AERD or AT control subjects reached confluence in culture more rapidly than did fibroblasts from healthy control subjects. We verified that the basal rates of proliferation were higher for both groups of polyp fibroblasts (Figure 1A). Despite their rapid proliferative rate, AT control

fibroblasts were significantly more responsive to growth suppression by PGE₂ than were fibroblasts from patients with AERD or healthy control subjects. Fibroblasts from both groups of control subjects were markedly more responsive to the selective EP₂ receptor agonist ONO-AE1-259-01 than were AERD fibroblasts (Figure 1B). The fact that all groups responded to forskolin indicated that the differences in EP₂ receptor-dependent suppression were not due to defects in adenylate cyclase or EPAC downstream of EP₂. The fact that the responses to PGE₂ did not completely parallel those to ONO-AE1-259-01 likely reflects contributions from additional EP receptors. Consistent with these findings, EP₂ receptor protein expression was substantially lower on AERD fibroblasts than on AT and healthy control fibroblasts (Figures 2A–2C). We therefore focused on understanding the mechanism responsible for the EP₂ protein expression on nasal fibroblasts.

The inducible expression of COX-2 and mPGES-1 in response to stimuli such as IL-1 β permits markedly increased production of PGE₂ during inflammation and physiologic stress. EP₂ and EP₄ receptors also exhibit inducible expression in many cell types. We found that resting fibroblasts expressed very low levels of EP₂ receptor mRNA (Figure 2D), and these levels were only weakly altered by stimulation with IL-1 β in control, AT, and AERD samples when compared with the synthetic enzymes, which demonstrated dramatic and similar fold induction in all groups (Figure 3A). The histone deacetylase inhibitor TSA functions broadly along the DNA to preserve acetylation and thereby maintains or increases the accessibility of promoters to transcription factors at sites that are suppressed due to a decrease in histone acetylation. In contrast to the other PGE₂ pathway genes, EP₂ receptor mRNA was significantly up-regulated by treatment of the fibroblasts with TSA, particularly in the cells from AT subjects and subjects with AERD (Figure 3B). Consistent with this observation, the levels of H3K27 acetylation at the *PTGER2* promoter were variable (Figure 4A) and correlated significantly to EP₂ receptor mRNA on fibroblasts from all subjects studied, independent of disease state (Figure 4B). In contrast, acetylation at *PTGER4* was constant across samples. Thus, variable

enrichment of H3K27Ac in the promoter region of *PTGER2* suggests that EP₂ receptor expression may be tightly regulated through a chromatin modification-dependent mechanism. This mechanism is one potential explanation for the impaired expression by fibroblasts from subjects with AERD. The fact that the AT control cells exhibit unimpaired EP₂ receptor function suggests contributions from additional regulatory steps, such as mRNA stability. Reduced EP₂ expression is also reported in nasal polyp inflammatory cells from subjects with AERD (16), which may contribute to the well-recognized feature of dysregulated leukotriene production. Further assessment of histone acetylation at the *PTGER2* promoter in other cell types of the nasal polyp is warranted to determine whether histone acetylation regulates EP₂ expression more globally in nasal polyps. This mechanism differs from the hypermethylation of CpG islands that restrains EP₂ receptor expression by fibroblasts from the lungs of subjects with pulmonary fibrosis (8) and may be relatively specific to nasal polyposis and AERD.

AERD is a disease state with identified clinical subphenotypes (18, 19). These subphenotypes may reflect multiple pathobiologic mechanisms relating to dysfunction along the pathway of arachidonic acid metabolism. Our data demonstrate that reduced EP₂ receptor expression results in resistance to the antiproliferative effects of EP₂ signaling in nasal polyp fibroblasts from subjects with AERD and support a role for EP₂ signaling in the aberrant growth of nasal polyp tissue. Additionally, we implicate histone acetylation at the *PTGER2* promoter as one mechanism that contributes to EP₂ expression in nasal fibroblasts. This may be relevant to explain the dramatic clinical phenotype of recalcitrant nasal polyposis in AERD. Our findings also support a contribution from epigenetic factors in the disease, which is consistent with the lack of heritability, adult onset, and irreversible nature of AERD. Such epigenetic mechanisms may contribute to the widespread abnormalities in arachidonic acid metabolism and lipid mediator receptor expression reported in AERD. ■

Author disclosures are available with the text of this article at www.atsjournals.org.

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