

Genetic and Epigenetic Components of Aspirin-Exacerbated Respiratory Disease



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KEYWORDS

- Cysteinyl leukotriene • Eosinophil • Biomarker • Epigenetics • Polymorphism
- AERD

KEY POINTS

- Aspirin-exacerbated respiratory disease (AERD) severity and its clinical phenotypes are characterized by genetic variation within pathways for arachidonic acid metabolism, inflammation, and immune responses.
- Epigenetic effects, including DNA methylation and histone protein modification, contribute to regulation of many genes that contribute to inflammatory states in AERD.
- The development of noninvasive, predictive clinical tests using data from genetic, epigenetic, pharmacogenetic, and biomarker studies will improve precision medicine efforts for AERD and asthma treatment.

INTRODUCTION

Aspirin intolerance is a severe and rare asthmatic endotype, with prevalence rates of 10% in the adult asthmatic population and up to 25% in patients with severe, persistent asthma.^{1–4} Consistent with the classification of asthma as a set of individual subtypes of diseases of varying symptoms and severity, AERD is distinguished from other types of severe asthma primarily by its clinical characteristics. The clinical features of AERD include airway obstruction, increased exacerbations, chronic rhinosinusitis, the presence of nasal polyps (NPs), eosinophilia, increased need for systemic glucocorticoids and poor response to asthma controller medication, and an increase in urinary leukotrienes (LTs), both in comparison to aspirin-tolerant asthma (ATA) and after aspirin challenge and symptom exacerbations.^{5,6} Due to the discovery

Disclosure Statement: The authors have nothing to disclose. This work was supported by grants from the National Institutes of Health and National Heart, Lung, and Blood Institute (U01HL065899-09 and K12 HL120004-02).

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Immunol Allergy Clin N Am 36 (2016) 765–789

<http://dx.doi.org/10.1016/j.iac.2016.06.010>

immunology.theclinics.com

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that increased production of LTs is a characteristic of AERD, the LT and prostaglandin (PG) production pathways were among the first to be investigated, and the subsequent identification of polymorphisms in LT-related genes in affected patients suggested a pivotal role for genetic variation in the development of AERD.^{6–8} As a result, variation in patient genetics has received considerable focus as a potential determinant of AERD pathogenesis.

The observation that severely asthmatic subjects responded favorably to antileukotriene asthma medications contributed further evidence toward a mechanistic role for LTs, while also providing an opportunity for clinicians to more appropriately tailor treatment to a specific patient group.^{7,9–12} Subsequent genetic studies revealed considerable evidence for genetic variation in AERD pathophysiology across multiple biological pathways^{7,13} as well as variation in interindividual treatment responses to multiple asthma drug classes, including leukotriene modifiers and inhibitors.¹⁴ The exact mechanisms by which LT synthesis becomes dysregulated in AERD, however, are still unknown. Due to corresponding alteration of immune molecules (eg, type 2 helper T cell [T_H2] cytokines), PGs (eg, PGE_2), and other inflammatory biomarkers (eg, interleukin [IL]-5, periostin, immunoglobulin (Ig) E, apolipoprotein A1, and others), multiple interacting pathways and mechanisms likely also contribute. Evidence that AERD has a heritable basis is minimal, and only two studies reported that 1% to 6% of individuals with AERD had an affected family member.^{4,15} The adult onset of AERD, combined with the low genetic penetrance and inconsistent replication of results from genetic associations, point toward involvement of environmental exposures and epigenetic factors in its progression. Achieving a better understanding of the genetic and epigenetic determinants of heterogeneity of AERD through genome-wide and epigenome-wide interrogation is, therefore, anticipated to improve strategies to develop more precisely tailored therapeutic agents, treatment regimens, and potentially cures for the disease.

UPDATE ON THE GENETICS OF ASPIRIN-EXACERBATED RESPIRATORY DISEASE

The quest to discover determinants of AERD (and its unique clinical features) has yielded a rapidly increasing number of candidate gene and genetic association studies. These studies reveal mechanistic insights into the molecular pathways for aspirin hypersensitivity, including arachidonic acid metabolism and cysteinyl leukotriene (CysLT) production, inflammatory cascades initiated by eosinophils, mast cells, platelets, airway epithelial cells, and others. For reference, this article summarizes the major results from these studies in [Table 1](#). Findings from many of these studies are conflicting, however, and a majority of reported associations lack replication. This section provides a comprehensive update of the status of genetic investigations of aspirin-sensitive asthma and AERD, highlighting major discoveries published within the past several years. In addition to discussing genetics association studies of AERD risk, recent findings are presented from investigations of genetic markers associated with two predominant AERD clinical features—nasal polyposis² and eosinophilia.⁶

Genetic Markers Associated with Disease Status and Clinical Features of Aspirin-Exacerbated Respiratory Disease

Aspirin-exacerbated respiratory disease susceptibility

Previous studies have yielded a substantial number of genes and genetic markers associated with AERD affection status and/or clinical phenotypes (summarized in [Table 1](#)). This section discusses recent discoveries with compelling evidence for a role in AERD pathogenesis.

Table 1
Summary of results from genetic studies of aspirin-sensitive asthma

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s), or Functional Effect(s)	Study Population(s) and Ethnicities ^a	Replication Population(s) and Ethnicities ^a	Study Type	References (PMID)
Airway cell function and response	<i>ADRB2</i>	rs1042713	Reference homozygous genotype is more common in ATA vs AERD; no effects on lung function measures or IgE	95 AERD; 300 ATA; 100 NC; Asian (Japanese)		CGAS	21829036
	<i>EMID2</i>	EMID2_BL2_ht2	Differences in Δ FEV ₁ by aspirin provocation in AIA vs ATA	163 AIA; 429 ATA; Asian (Korean)		CGAS	21086123
	<i>KIF3A</i>	rs3756775	Associated with the rate of FEV ₁ decline by aspirin provocation in AIA	103 AIA; 268 ATA; Asian (Korean)		CGAS	20922562
	<i>SPINK5</i>	G1258A, A1103G	Heterozygous genotypes were more frequently observed in AIA	15 CR5sNP; 59 CR5wNP (18 AIA); 30 NC; white (European)		CGAS	22570283

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Table 1
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Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s), or Functional Effect(s)	Study Population(s) and Ethnicities ^a	Replication Population(s) and Ethnicities ^a	Study Type	References (PMID)
Arachidonic acid metabolism and signaling	<i>LTC4S</i>	−444A > C	Association with ATA but not AIA	356 AIA; 840 ATA; 902 NC; Multiple (white, Asian, African American)		MA	22884858
	<i>ALOX5</i>	ht1[GCGA]	Increased frequency of haplotype for AIA	93 AIA; 181 ATA; 123 NC; Asian (Korean)		CGAS	14749922
	<i>COX2</i>	−765G > C	CC homozygosity was associated with disease severity; increased PG production by monocytes	112 AIA; 198 ATA; 547 NC; white (Polish)		CGAS	15316498
	<i>CYP2C19</i>	rs4244285, rs4986893	Lower % predicted FEV ₁ after aspirin provocation in AERD	100 AERD; 300 ATA; 100 NC; Asian (Japanese)		CGAS	21855977
	<i>CYSLTR1</i>	−634C > T, −475A > C, −336A > G	Higher frequency and promoter activity of ht(TCG) in AERD	105 AIA; 110 ATA; 125 NC; Asian (Korean)		CGAS	16630147
	<i>CYSLTR2</i>	−819T > G, 2078C > T, 2534A > G	Increased frequency of minor alleles and decline in FEV ₁ by aspirin provocation in AIA	86 AIA; 134 ATA; 152 NC; Asian (Korean)		CGAS	15970796
	<i>EP2</i>	u55, u55b, u57	Associated with AIA; reduced transcriptional activity of the EP2 gene	87 AIA; 192 ATA; 96 NC; Asian (Japanese)	198 AIA; 282 ATA; 274 NC; Asian (Japanese)	CGAS	15496426
	<i>NAT2</i>	−9246G > C	Associated with risk of AIA	170 AIA; 268 ATA; Asian (Korean)		CGAS	20602614
	<i>PTGDR</i>	−613C > T, −549T > C, −441C > T, −197T > C	Diplotype is more frequent in patients with aspirin triad, asthma and aspirin intolerance than NC	145 Asthma + NP; 81 AIA + NP; 75 aspirin triad + NP; 245 NC; white (Spanish)		CGAS	23101307
	<i>PTGER</i>	rs7543182, rs959	Associated with risk of AIA in the discovery population	137 AIA; 268 ATA; Asian (Korean)	106 AIA; 651 ATA; Asian (Korean)	CGAS	20587336

	<i>TBXA2R</i>	rs11085026	Increased association and greater percent fall of FEV ₁ after aspirin provocation for AIA	93 AIA; 172 ATA; 118 NC; Asian (Korean)	CGAS	15898979
	<i>TBXAS1</i>	rs6962291	Lower frequency of minor allele; association with fall of FEV ₁ after aspirin provocation for AIA	200 AIA; 270 ATA; Asian (Korean)	CGAS	21449675
Inflammatory responses	<i>ACE</i>	−262A > T, −115T > C	Increased risk of AIA; homozygotes for minor alleles had a greater decline in FEV ₁ after aspirin provocation than reference homozygotes	81 AIA; 231 ATA; 181 NC; Asian (Korean)	CGAS	18727619
	<i>AGT</i>	p2401C > G, p2476C > T	Increased MAF in AIA cases resistant to montelukast treatment; associated with modified response to montelukast	56 AIA; Asian (Korean)	CGAS	21624492
	<i>CACNG6</i>	rs192808; CACNG6_B11_ht6	Associated with increased risk of AIA	102 AIA; 429 ATA; Asian (Korean)	CGAS	20860846
	<i>CCR3</i>	−520T > C	Predictive accuracy for AIA >65% (with B2ADR 46A > G, CysLTR1−634C > T, and FCER1B−109T > C)	94 AIA; 152 ATA; Asian (Korean)	CGAS	18379861
	<i>CRTH2</i>	−446T > C	Serum eotaxin-2 was significantly higher in AERD with the TT genotype than CT/CC	107 AERD; 115 ATA; 133 NC; Asian (Korean)	CGAS	19796209
	<i>ECP</i>		mRNA and protein expression increased in AERD	15 AERD; 15 CRSwNP; unspecified (United States)	EP	26067893
	<i>ERAS</i>		mRNA expression was increased in AIA vs controls	11 AIA; 7 ATA; 15 NC; white (Polish)	EP	26646719
	<i>FOSL1</i>		mRNA and protein expression reduced in AIA vs controls	11 AIA; 7 ATA; 15 NC; white (Polish)	EP	26646719

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	<i>HSPA1B</i>	rs6457452, rs1061581	Increased MAF in AERD vs ATA; variance in eosinophil count by SNP for AERD vs ATA	102 AERD; 300 ATA; 100 NC; Asian (Japanese)		CGAS	23392055
	<i>IL13</i>	rs1800925	Higher MAF in AERD vs ATA	95 AERD; 300 ATA; 100 NC; Asian (Japanese)		CGAS	22123380
		–1510A > C	Reduced MAF in AIA vs ATA for AA genotype vs CC genotype of –1510A > C	162 AIA; 301 ATA; 430 NC; Asian (Japanese)			20358028
	<i>NOS2A</i>	[(CCTTT)n]	Association of more than 14 repeats of the NOS2A (CCTTT) repeat cluster in AIA and aspirin triad than NC	81 AIA; 75 aspirin triad; 245 NC; white (Spanish)		CGAS	23101307
	<i>PPARG</i>	82466C > T	Increased MAF in AIA vs ATA	60 AIA; 343 ATA; 449 NC; Asian (Korean)		CGAS	20224667
	<i>RAB1A</i>	14,444T > G, 41,170C > G	Increased MAF in AIA vs ATA, and association with FEV ₁ decline after aspirin provocation	181 AIA; 1016 ATA; Asian (Korean)		CGAS	24555545
	<i>SLC6A12</i>	rs557881	Associated with AIA and decline in FEV ₁ after aspirin provocation	163 AIA; 429 ATA; Asian (Korean)		CGAS	20597903
	<i>STK10</i>	rs2306961	Associated with AIA	163 AIA; 429 ATA; Asian (Korean)		CGAS	21905501

Initiation of immune responses	<i>CIITA</i>	rs1139564	Associated with nasal polyposis in AERD	158 AERD; 309 ATA; Asian (Korean)	CGAS	23292525
	<i>CNPY3</i>		mRNA expression reduced in AIA vs controls	11 AIA; 7 ATA; 15 NC; white (Polish)	EP	26646719
	<i>GM-CSF</i>		Elevated mRNA and protein in AERD	15 AERD; 15 CRSwNP; (United States)	EP	26067893
	<i>HLADPB1</i>	rs3128965	Increased MAF, bronchial hyperresponsiveness to inhaled aspirin and methacholine, and higher 15-HETE levels	179 AERD; 1989 HC; Asian (Korean)	264 AERD; 387 ATA; 238 NC; Asian (Korean)	GWAS 25536158
		rs1042151	Increased AERD susceptibility and gene dose effect for percent decline in FEV ₁ after aspirin provocation	117 AERD; 685 ATA; Asian (Korean)	142 AERD; 946 ATA; Asian (Korean)	GWAS 23180272
		exm537513	Increased risk of AERD; predictive for AERD susceptibility vs ATA	165 AERD; 397 ATA; 398 NC; Asian (Korean)		EWAS 25372592
		DPB1*0301	Increased MAF in AIA	59 AIA; 57 ATA; 48 NC; white (Polish)		CGAS 9179433
	<i>HLADPB2</i>	exm-rs3129294	Predictive for AERD susceptibility vs ATA	165 AERD; 397 ATA; 398 NC; Asian (Korean)		EWAS 25372592
	<i>HLADQA1</i>	DQA1*0301, DQA1*0201, DQA1*0501	Haplotypes associated with AERD risk	33 AERD; 17 ATA; 100 NC		CGAS 25975240
	<i>HLADQB1</i>	DQB1*0301	Lower MAF for AERD vs ATA	33 AERD; 17 ATA; 100 NC		CGAS 25975240
		DQB1*0302	Lower MAF in poor responders to aspirin desensitization	16 AERD		CGAS 26366802
	<i>HLADRB1</i>	DRB1*04, DRB1*07, DRB1*011	Haplotypes associated with AERD risk	33 AERD; 17 ATA; 100 NC		CGAS 25975240
	<i>HLADRB3</i>		MAF lower for AERD vs ATA	33 AERD; 17 ATA; 100 NC		CGAS 25975240
	<i>HLADRB4</i>		MAF higher for AERD vs ATA	33 AERD; 17 ATA; 100 NC		CGAS 25975240
	<i>IL10/TGFβ</i>	–1082A > G, –509C > T	–1082A/G associated with AIA; synergistic effect between TGF- β 1 –509C/T and IL-10–1082A/G in AIA	173 AIA; 260 ATA; 448 NC; Asian (Korean)		CGAS 19222424

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	<i>IL17RA</i>	–1075A > G, –947A > G, –50C > T, BL1_ht1	MAF lower for AERD vs ATA	143 AERD; 411 ATA; 825 NC; Asian (Korean)		CGAS	23220496
	<i>IL4</i>	–589T > C, –33C	Both associated with AIA risk; MAF for –589T > C was higher in the AIA vs ATA and associated with a gene dose-dependent decline in FEV ₁ after aspirin provocation	103 AIA; 270 ATA; Asian (Korean)		CGAS	20921925
	<i>IL5RA</i>	–5993G > A	AA genotype had increased IgE responses to staphylococcal enterotoxin A in AERD patients	139 AERD; 171 ATA; 160 NC; Asian (Korean)		CGAS	23470716
	<i>MCP-1</i>		Elevated mRNA and protein in AERD	15 AERD; 15 CRSwNP; unspecified (United States)		EP	26067893
	<i>TAP2</i>	Multiple haplotypes	Associated with FEV ₁ decline by aspirin provocation in AERD	93 AERD; 96 ATA; Asian (Korean)		CGAS	21796142
	<i>TLR3</i>	–299698G > T, 293391G > A	Increased MAF in AERD vs ATA for –29969G > A; reduced MAF 2200698G > T in AERD vs ATA	203 AERD; 254 ATA; 274 NC; Asian (Korean)		CGAS	21461252
	<i>UBE3C</i>	rs3802122, rs6979947 rs10949635	Lower risk for AIA	163 AIA; 429 ATA; Asian (Korean)		CGAS	20934631
			Associated with nasal polyposis in ATA vs AERD	114 AERD; 353 ATA; Asian (Korean)		CGAS	21881582
Asthma/ NSAID response	<i>CEP68</i>	CEP68_ht4 (T-G-A-A-A-C-G), rs7572857	Associated with increased risk of AIA and higher decline of FEV ₁ after aspirin provocation	80 AIA; 100 ATA; Asian (Korean)	163 AIA; 429 ATA; Asian (Korean)	GWAS	21072201
	<i>DPP10</i>	rs17048175	Association with AERD but not ATA	274 AERD; 272 ATA; 99 NC; Asian (Korean)		CGAS	25592153

Abbreviations: A/G, A to G transition; C/T, C to T transition; CGAS, candidate gene association study; CRSwNP, chronic rhinosinusitis with nasal polyposis; CRSsNP, chronic rhinosinusitis without nasal polyposis; EP, expression profiling; EWAS, exome-wide association study; ht(TCG), haplotype TCG; MA, meta-analysis; NC, normal (non-asthmatic) controls; PMID, PubMed unique identifier; TGF, transforming growth factor.

^a Discovery/replication population and their race/ethnicity (if specified).

The best mechanistic evidence for AERD pathogenesis supports intrinsic dysregulation of the activity of the 5-lipoxygenase (LO)/LTC₄S pathway, leading to increased recruitment and tissue infiltration of immune effectors. These effects are mediated largely by alterations in genes that are directly involved in arachidonic acid metabolism and signaling, namely *LTC4S*,^{16,17} *ALOX5*,^{18,19} *CYSLTR1*,^{20–22} *CYSLTR2*,^{20,21} *TBX21*,²³ *EP2*,²⁴ and *COX2*.^{25,26} A summary of the clinical evidence for these associations is presented in **Table 1**. The arachidonic acid metabolism signaling pathway genes *LTC4S*, *ALOX5*, *CYSLTR1*, and *CYSLTR2* represent the most important candidate genes in this pathway and have the strongest evidence for a role in AERD pathogenesis. An *LTC4S*–444A/C promoter single nucleotide polymorphism (SNP) (rs730012) is among the most widely reported variants associated with AERD, although its association with AERD across studies is inconsistent.^{27–31} A recent meta-analysis of 13 case-control studies of asthma revealed significant increased risk in ATA populations carrying the CC or AC genotype versus AA genotype (odds ratio [OR] 1.36; 95% CI, 1.12–1.65; *P* = .002) but not in aspirin-intolerant groups (OR 1.16; 95% CI, 0.89–1.52; *P* = .27).³¹ Therefore, variation in *LTC4S*, although consistently associated with ATA, may not be consistently related to AERD across populations. Three *ALOX5* promoter variants have been associated with AERD and/or its severity of hyper-responsiveness^{19,32,33} (see **Table 1**). New *ALOX5* variants associated with AERD, however, have not been identified. The *CYSLTR1* and *CYSLTR2* leukotriene receptor genes are among the most important for leukotriene signaling and are pharmacologic targets for montelukast, the gold standard prescribed medication for AERD symptom control.^{34–36} *CYSLTR1* is overexpressed in nasal tissues of AERD patients, and 3 promoter SNPs in *CYSLTR1* have been associated with both AERD status and higher *CYSLTR1* promoter activity, suggesting that functional variation driving overexpression of this receptor underlies its pathologic roles in LT signaling in AERD^{37,38} (see **Table 1**). Polymorphisms in *CYSLTR2* are also associated with AERD and forced expiratory volume in the first second of expiration (FEV₁) decline after aspirin provocation test (see **Table 1**), suggesting a role for this receptor as well in driving clinical features of AERD.

In addition to these genes, novel candidate genes within the arachidonic acid pathway were recently evaluated for their association with AERD. Prior genetic studies demonstrated an association of asthma susceptibility with *dipeptidyl-peptidase 10* (*DPP10*), which encodes a potentially nonfunctional serine protease with unknown biological roles.^{39,40} The association was also correlated with serum DPP10 levels. This association, and correlation with serum DPP10, was replicated in a follow-up association study in 272 AERD patients, 272 ATA patients, and 99 healthy controls of Korean ethnicity.⁴¹ In addition, there was a significant correlation of serum DPP10 levels with the serum levels of 15-hydroxyeicosatetraenoic acid (HETE), an arachidonic acid pathway metabolite that is released at higher levels in eosinophils from severely affected AERD patients.⁴¹ Although the biological roles of DPP10 in asthma tolerant asthma are unclear, its increased serum protein levels and correlation with serum 15-HETE suggest that these may be protein biomarkers for AERD.⁴¹ Another arachidonic acid pathway gene, *FABP1*, was suspected of involvement in AERD due to its roles in regulating bioactive lipid mediators; however, no significant association between the *FABP1* polymorphisms and AERD or lung function was found.⁴² There is reasonably strong evidence implicating LTs and DPP10 in AERD pathogenesis. What remains unclear is what role genetic susceptibility plays in disease onset and whether other pathways are involved in disease pathogenesis.

Genetic associations with nasal polyposis in aspirin-exacerbated respiratory disease/aspirin-induced asthma

AERD comprises up to 30% of asthmatics with NPs.^{25,43} Inflammatory mediators in the T_H2 cytokine pathway may drive the development of symptoms characteristic of AERD, including chronic rhinosinusitis associated with nasal polyposis.^{43–45} Patients with AERD undergo a greater frequency of revision sinonasal surgeries and have a higher rate of postsurgical symptomatic recurrence than patients with non-AERD-related chronic rhinosinusitis with NPs.^{44–46} The genetic and molecular mechanisms, however, that can differentiate this particular AERD phenotype from non-AERD phenotypes with nasal polyposis are unclear. Comparison of an inflammatory response signature, including T_H2 and non- T_H2 cytokine and chemokine-encoding genes, identified from microarray expression profiling of inflammatory mediators within NP samples from patients with chronic rhinosinusitis versus patients with AERD, revealed significantly elevated expression of 5 mediators (eosinophilic cationic protein [ECP], GM-CSF, *SDF-1 α* and *SDF1 β* , MCP-1, and *IL10*) and reduced expression of tissue plasminogen activator in the NPs of AERD.⁴⁷ AERD NPs also contained significantly elevated protein levels of ECP, GM-CSF and MCP-1 compared with the chronic rhinosinusitis samples,⁴⁷ as well as increased eosinophilia. No corresponding increase in T_H2 -specific protein expression was associated, however, with eosinophil proliferation and recruitment in AERD samples, suggesting that other non- T_H2 processes, may be important for AERD pathogenesis.⁴⁷

A major histocompatibility complex (MHC)-related gene, *class II MHC transactivator (CIITA)*, is expressed in NPs, and polymorphisms in this gene are associated with the development of multiple immune-related disorders due to the importance of MHC genes in regulating immune responses.⁴⁸ Eighteen *CIITA* SNPs hypothesized to play a role in AERD were genotyped in 158 AERD patients and 309 ATA patients of Korean ancestry, and one SNP, rs1139564, was nominally associated with NPs in the AERD group.⁴⁸ This association did not persist, however, after multiple test corrections.⁴⁸

Because eicosanoids and their receptors are up-regulated in inflammatory cells within NP tissue,^{49,50} corresponding to high levels of *LTC4S*,⁵¹ *CYSLTR1*,²⁰ and *PTGDR*,²⁵ transcript expression in NPs, altered leukotriene metabolism is also implicated in the development of this phenotype in AERD. A candidate gene study of variants in *LTC4S* (–444A > C), *PTGDR* (–613C > T, –549T > C, –441C > T, and –197T > C), *CYSLTR1* (927T > C), and *NOS2A* [(CCTTT)*n*] in samples from 81 asthmatics with nasal polyposis and aspirin intolerance, 75 patients with nasal polyposis and the aspirin triad, and 245 unaffected controls, revealed a significant association for more than 14 repeats of the *NOS2A* (CCTTT) repeat cluster in patients with aspirin intolerance (OR 3.68; 95% CI, 1.31–10.36; *P* = .009) and in patients with the aspirin triad (OR 0.25; 95% CI, 0.09–0.72; *P* = .005).⁵² In addition, the *PTGDR* diplotype CCCT/CCCC (–613CC, –549CC, –441CC, and –197TC) occurred more frequently among patients with the aspirin triad (OR 3.16; 95% CI, 1.05–9.49; *P* = .04).⁵² Nitric oxide is an important inflammatory mediator produced at high levels during inflammatory states that is carried out predominantly by *NOS2A* in the paranasal sinuses.⁵³ Modification of *NOS2A* transcript expression may be crucial for development of NPs,⁵⁴ pointing toward an important role for this gene in development of this phenotype.

In summary, genetic studies of nasal polyposis in AERD/aspirin-induced asthma (AIA) implicate inflammation and the eicosanoid pathway.

Genetic associations with eosinophilia in aspirin-exacerbated respiratory disease/aspirin-induced asthma

Persistent eosinophilia and cytokine overproduction are critical clinical features of AERD⁴⁷; furthermore, eosinophil activation and migration require the presence of

cytokines and other immune mediators. Eosinophilia is a T_H2 cytokine-dependent process, and expression of IL-4, IL-5, IL-13, and other cytokines correlates with eosinophilic infiltration.^{43,47} T_H2 cytokine IL-5 receptor alpha (*IL5RA*) polymorphisms have been reported in asthma and allergic diseases and are associated with increased levels of peripheral blood eosinophils, although a direct association with AERD has not been clarified. In a recent study to determine whether *IL5RA* polymorphisms were involved in eosinophil activation in AERD, 139 AERD patients, 171 ATA patients, and 160 normal controls of Korean ancestry were genotyped for 3 suspected *IL5RA* SNPs (−5993G > A, −5567C > G, and −5091G > A) and a case-control analysis and functional characterization of the SNPs were performed.⁵⁵ AERD patients with *IL5RA* −5993AA demonstrated a higher IgE to staphylococcal enterotoxin A ratio than heterozygotes or those possessing the reference allele.⁵⁵ Furthermore, −5993A demonstrated altered promoter activity by luciferase reporter assay and differential binding of nuclear extracts by EMSA.⁵⁵ The investigators conclude that *IL5RA* −5993G > A may, therefore, contribute to eosinophil responses in AERD patients.⁵⁵

In addition to IL5, evidence exists for the recently described IL-17 cytokine family in inflammatory cell recruitment and allergic response.^{56,57} Polymorphisms in the IL-17A receptor gene, *IL17RA*, are associated with asthma, and IL-17A activation induces activation of signaling molecules, such as nuclear factor (NF)- κ B, that regulate inflammatory processes in human airway cells.⁵⁸ In a recent candidate gene study in a Korean population, 15 SNPs in *IL17RA* were analyzed and functionally characterized in 143 patients with AERD, 411 patients with ATA, and 825 normal controls.⁵⁹ Three *IL17RA* SNPs (−1075A > G, −947A > G, and −50C > T) were significantly associated with the risk of aspirin intolerance as well as the rate of decline in FEV₁ after aspirin challenge, although the minor allele frequencies (MAFs) for all 3 SNPs were significantly lower for AERD.⁵⁹ Finally, *IL17RA* expression in CD14⁺ monocytes from asthmatic patients with all 3 minor allele genotypes for *IL17RA* −1075A > G, −947A > G, and −50C > T was significantly higher than for the reference homozygotes.⁵⁹ The minor alleles of the 3 SNPs may, therefore, have protective effects for AERD, presumably by limiting *IL17RA* expression.⁵⁹

The MHC II HLA locus is involved in T-cell activation and has been shown in multiple genetic investigations to have strong associations with asthma. To date, the best genetic marker for AERD is *HLADPB1*0301*, which is also associated with a higher leukotriene receptor antagonist dose to control symptoms and a higher prevalence of chronic rhinosinusitis.^{60–62} A recent genetic association study of *HLA-DRB*, *HLA-DQA1*, and *HLA-DQB1* genotypes in 33 patients with AERD, 17 patients with ATA, and 100 healthy controls was performed after an oral aspirin challenge.⁶³ In comparison to the controls, frequencies of *HLA-DQB1*0302* and *HLA-DRB1*04* and the haplotypes *HLA-DRB1*04/DQA1*0301/DQB1*0302* and *HLA-DRB1*07/DQA1*0201/DQB1*0201* were higher in patients with AERD whereas *HLA-DQB1*0301*, *HLA-DQA1*0501*, *HLA-DRB1*11*, and *HLA-DRB3* allele frequencies were significantly lower.⁶³ Furthermore, in contrast to ATA patients, patients with AERD had lower frequencies of *HLA-DQB1*0301* and *HLA-DRB1*011*.⁶³

The infiltration of eosinophils characteristic of AERD is also promoted by their release of CysLTs. In contrast to ATA with eosinophilic sinusitis, AERD patients show increased expression of leukotriene receptors and hyper-reactivity to CysLTs.⁴³ Distinguishing the characteristics of eosinophilia occurring in AERD from eosinophilia occurring in ATA may shed light on unique cellular and molecular features of the disorders, facilitating greater understanding of these endotypes, and thus provide opportunities for more precise treatment. Steinke and colleagues⁶⁴ profiled the expression of type 1 helper T cell (T_H1) and T_H2 cytokines in tissue samples

from 30 asthmatics with chronic hyperplastic eosinophilic sinusitis, 15 patients with AERD, and 9 healthy controls, using quantitative real-time polymerase chain reaction.⁶⁴ The investigators determined that although a T_H2 cytokine signature, as shown by increased expression of IL-4, IL-5, and IL-13, compared with control tissue dominated in both AERD and ATA, AERD alone demonstrated overexpression of T_H1 cytokine interferon (IFN)- γ , which was also determined as originating from eosinophils through flow cytometry and histologic studies.⁶⁴ Furthermore, in addition to priming these eosinophils, IFN- γ increased the expression of genes involved in leukotriene biosynthesis and CysLT secretion.⁶⁴ IFN- γ increases CysLT receptor expression on circulating eosinophils and also increased *LTC4S*, *CYSLT1*, and *CYSLT2* mRNA expression in both mature eosinophils as well as their progenitors, coincident with a significantly enhanced capability of eosinophils to degranulate and secrete CysLTs after stimulation with IFN- γ .^{64,65} These findings suggest that IFN- γ may uniquely drive the CysLT overproduction in AERD and may represent an important diagnostic marker.

Additional genes that may regulate proinflammatory cellular responses, including the Ras oncogenes and heat shock proteins (HSPs), have also been recently investigated. *RAB1A* encodes a Ras family GTPase that may contribute to eosinophilia and immune responses in AERD by regulating vesicle exocytosis in activated immune cells. Eight polymorphisms in the *RAB1A* gene were analyzed for associations with the risk of AERD in 181 asthmatic subjects with aspirin intolerance and 1016 non-aspirin-intolerant asthmatic controls.⁶⁶ Two SNPs, *RAB1A* 14444G > T and *RAB1A* 41170C > G, were associated with aspirin hypersensitivity and the MAFs of these SNPs were also higher in this group.⁶⁶ In addition, the *RAB1A* 14444TT and *RAB1A* 41170GG carriers showed a greater decline in FEV₁ after oral aspirin challenge, with heterozygotes for both SNPs demonstrating intermediate levels of FEV₁ decline.⁶⁶ The Ras family GTPases regulate production of inflammatory mediators,⁶⁷ and granule release from platelets, eosinophils, and neutrophils requires *RAB1A* activation.⁶⁸ These data suggest that *RAB1A*, and potentially other members of the Ras oncogene family, may therefore contribute to the development of AERD and merit further investigation.

The HSP family members, in particular HSP70, are correlated with asthma severity and eosinophilia and overexpressed in the asthmatic airway.^{69,70} The role of HSPs in allergic asthma, however, is unclear. To investigate the hypothesis that HSP70 variation contributes to AERD susceptibility, a recent study evaluated the association of 2 candidate HSP70 polymorphisms—*HSPA1B*-179C > T and *HSPA1B*-1267A > G (rs6457452 and rs1061581)—in 102 asthmatics with AERD, 300 asthmatics with ATA, and 100 normal healthy controls, all of Japanese descent.⁷¹ In patients with AERD, compared with the ATA group, frequency of the *HSPA1B*-179CT/TT genotype was higher than that of the CC genotype, and the GG genotype of the *HSPA1B*-1267GG was also higher than that of the GA/AA genotype; furthermore, frequency of the *HSPA1B*-179C/1267A haplotype was also higher in the AERD group versus the ATA group and was associated with a significantly increased risk of AERD (OR 3.154; 95% CI, 1.916–5.193).⁷¹ Finally, AERD patients demonstrated a significant variation in eosinophil count by *HSP* SNP genotype, whereas the aspirin-tolerant group did not.⁷¹ Although the molecular mechanisms of HSP70 variation and eosinophilia in AERD were not investigated in this study, the investigators suggest that, as a possible mechanism, because the HSP-encoding genes are located within the MHC III region,⁷² the HSP70 SNPs may be in linkage with other SNPs within this region, which is also adjacent to *TNF*, and that could functionally contribute to this association.⁷¹

Genome-wide Approaches for Investigating Genetic Relationships in Aspirin-Exacerbated Respiratory Disease

To date, multiple genetic risk factors for AERD have been identified through candidate gene studies and genome-wide association studies (GWAS) (see [Table 1](#)). The latest GWAS of AERD, conducted in 2014, analyzed 2379 subjects and also replicated initial findings in an independent cohort of 264 AERD patients, 238 healthy controls, and 387 patients with ATA.⁷³ Using the Affymetrix Genome-Wide Human SNP Array, Kim and colleagues⁷³ profiled 275,862 SNPs from 179 AERD patients, 211 patients with aspirin-exacerbated cutaneous disease (AECD), and 1989 healthy control subjects. Although none of the SNP associations achieved genome-wide significance, rs3128965 in *HLA-DPB1* approached genome-wide significance and was associated with AERD in both the discovery and replication populations.⁷³ Furthermore, asthmatic patients carrying the minor allele of this SNP demonstrated significantly enhanced bronchial hyper-responsiveness to aspirin and methacholine, in addition to higher 15-HETE levels.⁷³ These data suggest that rs3128965 could represent a potential diagnostic genetic marker for AERD. A prior GWAS had also identified a SNP in *HLA-DPB1* (rs1042151) associated with AERD.⁷⁴ In this study, 430,486 SNPs were analyzed for association with AERD, using the Human660W BeadChip (Illumina), in 117 subjects with AERD and 685 ATA patients.⁷⁴ None of the SNPs achieved genome-wide significance; however, rs2281389 near *HLA-DPB1* was most strongly associated with AERD (OR 2.41; $P = 5.69 \times 10^{-6}$).⁷⁴ The top 49 SNPs associated with AERD risk were also associated with significant decline of FEV₁ after aspirin challenge.⁷⁴ For replication, 702 SNPs in the 14 genes were genotyped in 142 AERD and 996 ATA subjects, and a nonsynonymous SNP in *HLA-DPB1*, rs1042151, showed the highest association with the risk of AERD.⁷⁴ For reference, the results of earlier GWAS of AERD and AIA are provided in [Table 1](#).

Although candidate gene studies have yielded a wealth of information on AERD genotype-phenotype associations, these hypothesis-driven approaches necessarily focus on a small number of genes and, therefore, exclude loci that could also have direct functional importance for the phenotype. Comparing gene expression and whole-sequence profiles from AERD cases and non-AERD asthmatics or healthy controls using whole-genome microarray expression profiling and next-generation sequencing methods provides a discovery-based approach to interrogate mRNA transcripts across the genome with specific correlation to the phenotype. A combined approach using microarray expression profiling and a candidate gene analysis in peripheral blood mononuclear cells (PBMCs) from a small white population identified 3 genes with expression profiles that significantly differed between AIA versus ATA and/or AIA versus healthy subjects.⁷⁵ In particular, expression of *CNPY3* and *FOSL1* was significantly lower in AIA versus healthy controls, whereas *ERAS* expression was increased. Protein expression of *FOSL1* in PBMCs was also significantly lower for AIA than the control groups.⁷⁵ Although the study lacked mechanistic investigation of these novel candidate genes for AERD, the investigators suggest these genes could participate in innate immune response pathways and pathways for tissue/cell remodeling and airway hyper-responsiveness that contribute to the pathogenesis of AERD.⁷⁵

Genomic studies of complex diseases are increasingly focusing on elucidating the impact of coding variants, which are more likely to be rare and to have larger effect sizes corresponding to their functional significance for gene expression. Shin and colleagues⁷⁶ recently used an exome-wide profiling approach using the HumanExome BeadChip v1.1 (Illumina) to identify novel, rare, and exonic SNPs associated with

AERD status in 165 AERD patients, 397 patients with ATA, and 398 normal controls of Korean ancestry. After filtering and quality control of genotype data, more than 54,000 SNPs remained and were evaluated for association with AERD risk.⁷⁶ A SNP in *HLA-DPB1*, exm537513, achieved genome-wide significance and was associated with increased risk of AERD (OR 3.28; P value of 3.4×10^{-8}).⁷⁶ From the top 100 SNP associations, the P values of remaining top 10 SNPs ranged from 3.4×10^{-8} to 2.4×10^{-4} with ORs from 0.13 to 13.61.⁷⁶ Three additional exonic SNPs on *HLA-DPB1* (exm537513, exm537522, and exm537523) were also present among the top 20 SNPs. A prior GWAS⁷⁷ had identified exm537522 (also annotated as rs1042151 in that study) as having the strongest association with AERD susceptibility; therefore, the investigators replicated one of their top associations from a previous study.⁷⁶ To develop a predictive model for AERD risk, the investigators selected the best combination of the top 10 SNPs that could discriminate between AERD and ATA, using multiple logistic regression, and calculated receiver operating characteristic (ROC) curves and area under the curve (AUC) values for each combination model.⁷⁶ A combination model of 7 SNPs (exm537513, exm83523, exm1884673, exm538564, exm2264237, exm396794, and exm791954) in *HLA-DPB1*, *HLA-DPA1*, and *HLA-DPB2* could predict AERD versus non-AERD status (AUC of 0.75; 34% sensitivity and 93% specificity).⁷⁶ A major limitation of this study is that no replication was performed. In summary, GWAS tend to support the involvement of the HLA locus in the pathogenesis of AERD.

Limitations of These Studies

A significant limitation of genetic studies of AERD is that few have replicated their associations. Moreover, a majority of the top associations lacked genome-wide or experiment-wide significance. Furthermore, the lack of uniformity in genetic associations across diverse populations confounds generalizability of these loci to the AERD patient population. A consistent limitation of these, and clinical genetic studies in general, is the limited numbers of patients available for study, which greatly limits statistical power to detect actual SNP associations with phenotype. Genome-wide association studies also typically exclude rare variants (MAF <1%) that are more likely to be present in coding regions and have more direct correlations with function, limiting the ability to detect functional associations. Finally, a majority of studies also lack experimental validation of their genetic findings, limiting the ability to discern the molecular function of these variations and their potential clinical consequences. Future genetic studies of AERD must consider replication of initial findings across well-powered populations and include functional validation in appropriate cellular models.

EPIGENETICS OF ASPIRIN-EXACERBATED RESPIRATORY DISEASE

Epigenetic modifications include methylation of CpG islands in gene promoter regions, and the acetylation and deacetylation of histone proteins, all of which can significantly alter chromatin unfolding and hence gene expression. Furthermore, epigenetic modification patterns can vary greatly across cell and tissue types. There is a correlation between the rise in asthma susceptibility and early exposure to environmental allergens during development, which is potentially mediated through epigenetic mechanisms.⁷⁸ Through modifying gene expression, epigenetic changes can thereby alter phenotypes and direct adaptation toward survival during periods of environmental stress.

Few studies to date have investigated the roles of epigenetics in AERD. In the following sections, insights from recent studies of epigenetic modifications in AERD versus other allergic asthma endotypes are discussed.

Global Investigations of Epigenetic Modifications in Aspirin-Exacerbated Respiratory Disease

Given the dynamic regulation of expression of genes within immune and leukotriene response pathways and the corresponding lack of specific genetic markers that can explain the totality of the heterogeneity of these expression patterns, epigenetic modifications of the genome are probable contributors to AERD pathogenesis. A hallmark of epigenetic regulation is its tissue specificity, which generates specific gene expression profiles in different airway cell and tissue subsets. As discussed previously, NPs are a dominant clinical feature of AERD, are marked by eosinophilic migration and infiltration, and therefore may represent an ideal tissue model for the investigation of pathogenic cellular processes unique to AERD. A 2011 study investigated genome-wide DNA methylation levels in the context of aspirin-sensitive asthma in blood and NP samples from 5 patients with AIA and 4 patients with ATA.⁷⁹ DNA methylation profiles were interrogated using the Illumina genome-wide methylation assay chip.⁷⁹ Methylation of a total of 332 CpG sites in 296 genes was significantly increased among the patients with AIA compared with the patients with ATA, whereas 158 sites in 141 genes were significantly decreased; buffy coat DNA methylation patterns were not significantly diverse between the 2 groups.⁷⁹ Pathway analysis of the hypomethylated genes indicated enrichment in proliferation and activation of immune cells, cytokine production, and immune and inflammatory responses.⁷⁹ Alteration of these pathways through differential gene regulation may account for the spread of inflammation along the airways and proliferation of sinonasal cells leading to development of nasal polypsis. In particular, methylation patterns for 4 genes (*PGDS*, *ALOX5AP*, *PTGES*, and *LTB4*) that drive the arachidonic acid metabolism pathway that is uniquely dysregulated in AERD were altered; *PGDS* and *ALOX5AP* were hypomethylated, whereas *PTGES* was hypermethylated, suggesting that altered methylation patterns regulating expression of these genes could underlie aspirin hypersensitivity.⁷⁹ In addition, 2 T_H2 cytokine-encoding genes, *IL5RA* and *IL10*, were differentially methylated.⁷⁹ These data provide evidence that differences in gene regulation for arachidonic acid metabolism and immune response genes expressed in the upper and lower airway may account for the phenotypic differences observed between AIA and ATA.

In asthma and allergy, B lymphocytes are crucial regulators of adaptive and humoral immune responses and IgE production, which is a biomarker for hypersensitivity reactions. Furthermore, epigenetic patterns in B lymphocytes tend to be less variable across populations, which makes them a robust cellular model for comparative investigations of hypersensitivity related to allergy and asthma. Genome-wide DNA methylation profiles in CD19⁺ B lymphocytes from a small sample of allergic asthmatics and type I hypersensitive patients were compared with profiles from patients diagnosed with AERD, bronchial asthma, and healthy controls, and the initial results were validated in an independent population.⁵⁴ DNA methylation patterns in B lymphocytes from AERD patients and healthy controls showed greater concordance than those of allergic asthmatic subjects, presumably due to the greater degree of IgE production within a specific B-cell subset in the latter group.⁷⁹

Functional Epigenetics of Aspirin-Exacerbated Respiratory Disease

The role of epigenetic targeting of PGE₂ pathway genes involved in the expansion of NPs in AERD patients was recently investigated.⁸⁰ Fibroblasts, which are the major effector cells for airway remodeling, express the arachidonic acid pathway genes that are up-regulated in AERD, and stimulation of the prostaglandin E2 receptor 2 (EP₂) by PGE₂ represses the activation and growth of these cells. Prior evidence

from an epigenome-wide association study revealed that *PTGES*, the gene encoding microsomal PGE synthase-1 that converts PGH₂ to PGE₂, was hypermethylated in NP tissue from AERD subjects.⁷⁹ In addition, fibroblasts from NPs of patients with AERD have intrinsically lower expression of cyclooxygenase (COX)-2, PGE₂, and EP₂ receptor protein versus aspirin-tolerant control subjects.⁸¹ Cahill and colleagues⁸⁰ hypothesized that an intrinsic defect in EP₂ expression in NP fibroblasts, potentially a result of epigenetic modification at this locus, underlies the aggressive expansion and proliferation of NPs in AERD patients. To investigate this, the investigators first isolated and cultured fibroblasts from NPs of 18 patients with AERD and 9 aspirin-tolerant patients with chronic rhinosinusitis and nasal polyposis, and nasal tissue from 8 nonasthmatic controls undergoing surgery for concha bullosa.⁸⁰ In contrast to ATA, fibroblasts from AERD patients proliferated quickly and also demonstrated persistent growth in response to treatment with PGE₂ as well as having reduced expression levels of the EP₂ receptor.⁸⁰ In addition, in AERD samples, EP₂ receptor mRNA was significantly up-regulated by treatment of the fibroblasts with the histone deacetylase inhibitor trichostatin A, and histone acetylation (H3K27ac) at the EP₂ promoter correlated strongly with baseline EP₂ mRNA expression levels.⁸⁰ DNA methylation at the EP₂ promoter in fibroblasts, however, was not significantly different, suggesting that histone modification was more likely to contribute to EP₂ expression in nasal fibroblasts in AERD.⁸⁰ Together, these findings support a role of epigenetic effects in AERD.

Limitations of These Studies

There is a dearth of epigenetic investigations in AERD. A limitation of the reviewed studies is the lack of replication and poor statistical power due to small sample sizes investigated. Furthermore, to date, only a single well-designed study pursued functional characterization of specific epigenetic modifications in a cellular model of AERD. Additional epigenome-wide association studies focusing on replication, and detailed functional validation studies, are needed to clarify the extent to which specific epigenetic mechanisms contribute to AERD pathogenesis. These would be particularly informative with regard to aspirin challenge and at disease inception.

IMPACT OF GENETICS IN THE CLINICAL MANAGEMENT OF ASPIRIN-EXACERBATED RESPIRATORY DISEASE

LTs are bioactive lipids derived from arachidonic acid (AA) that serve as immunologic mediators.^{82–86} In AERD, LT overproduction has serious consequences for symptom severity and progressive airway disease.^{87,88} AERD patients show significant reductions in lung function, as determined by measuring FEV₁, compared with non-AERD asthmatics, and significantly higher baseline and postaspirin levels of urinary LTE₄, the final metabolite of CysLTs, corresponding to both the severity of respiratory disease and the up-regulation of CysLTR1 expression on inflammatory cells.⁸⁹ In addition, COX-1 inhibitors (including aspirin) remove a brake on 5-LO activation, thereby increasing the baseline overproduction of LTs in AERD patients.⁸⁸ AERD patients tend to require larger doses of asthma controller medications, and treatment with LT antagonists and inhibitors (zileuton, montelukast, and zafirlukast) improves symptoms in asthma and AERD patients. These medications are routinely prescribed in higher doses to prevent or attenuate bronchospasm in AERD patients, having a positive impact on AERD treatment and improving the safety of aspirin challenges.⁹⁰ The gold standard for diagnosis of AERD is oral aspirin challenge to provoke symptomatic response.⁹¹ During this response, CysLT production is dramatically increased, precipitating symptoms.^{88,89,91,92} After diagnosis, significant

improvement in asthma symptoms and slowing of NP recurrence are achieved with aspirin desensitization and daily high-dose aspirin treatment.⁹⁰ The dramatic increase in LT production immediately after aspirin challenge, and improved treatment response to montelukast in this population, provides a compelling rationale for extending investigations of LT pathway modulation in asthma to AERD. To this end, AERD represents an excellent clinical model of LT overproduction leading to a proinflammatory state to inform understanding of LT biology and treatment response.

Pharmacogenetics

Several pharmacogenetic studies of treatment responses in AIA have been performed. Recent investigations are discussed. Due to its ability to inhibit LT-mediated airway inflammation by blocking CysLT1 receptors, montelukast treatment ideally might benefit specific asthmatic patient subgroups with overproduction of LTs as a clinical feature, including patients with aspirin hypersensitivity.⁹³ Montelukast as monotherapy or add-on therapy is efficient in controlling asthma and allergic rhinitis in patients with poorly controlled asthma who require corticosteroids and/or long-acting β_2 -agonists.⁹³ Candidate gene and GWAS of anti-LT responses implicate involvement of multiple genes, including *ALOX5*,³² *ALOX5AP*,^{34,35,94–96} *LTC4S*,^{30,34–36,87,97} *CYSLTR1*,^{34–36,87} *CYSLTR2*,^{34,97} *ABCC1*,^{35,36,97–99} and *OATP2B1*.¹⁰⁰ Recently, the authors conducted the first pharmacogenomics GWAS of zileuton¹⁰¹ and montelukast¹⁰² responses in asthmatics and identified novel loci uniquely associated with both medications. Candidate gene studies of differential gene expression between non-AERD and AERD asthmatics also implicate multiple immune response and LT pathway genes, including the *HLA* allele *DPB1*,^{7,74,103} *CYSLTR1*,^{7,103} and *RGS7BP*.¹⁰⁴ These findings implicate involvement of multiple genes within, and related to, the LT pathway in regulating differential responses to treatment in asthma and AERD.

A pharmacogenetic study was recently conducted with the goal of identifying prognostic factors for AERD using clinical and genetic data associated with AERD according to the clinical course of disease and response to symptom control by corticosteroids, long-acting β -agonists, and antileukotrienes.¹⁰⁵ A total of 122 patients with AERD were classified according to symptomatic response to aspirin rechallenge after 1 or more years of treatment with asthma controller medications; group I patients (N = 48) had negative conversions to follow-up lysine-aspirin bronchoprovocation test whereas group II patients (N = 74) showed positive responses or persistent asthma symptoms.¹⁰⁵ DNA samples from peripheral blood were obtained from these individuals and a case-control genetic association study of 11 candidate loci in the leukotriene and inflammatory pathways (*ALOX5* 1708G > T, *ALOX15* 427G > A, *CCR3* 520T > G, *CRTH2* 466T > G, *CYSLTR1* 634C > T, *IL10* 1082A > G, *IL13* 1055C > T, *LTC4S* 444A > C, *TGF β* 509C > T, *TNFA* 308G > A, and *HLADPB1**0301) was conducted.¹⁰⁵ There were no significant differences in genotype frequencies between the 2 groups, with the exception of *CCR3*, for which the frequency of the G allele was significantly lower in group 1 than group II.¹⁰⁵ A significant, genotype-dependent relationship to conversions and responses was observed, with 61% of individuals carrying *CCR3* 503TT showing negative conversions at follow-up and 28.6% of the patients with GT or GG genotype demonstrating negative responses, increased incidence of nasal polyposis, and a greater decline in FEV₁ both at baseline and after lysine-aspirin bronchoprovocation test.¹⁰⁵ *CCR3* is a G-protein-coupled receptor that binds to several small chemoattractant proteins, known as CC-type chemokines, a class that includes the eotaxin family members that can direct eosinophils to inflammatory sites, and that are up-regulated in NP tissue.¹⁰⁵ These data suggest that the G allele of

CCR3 503 T > G is a genetic marker that can predict persistent aspirin hypersensitivity and, by virtue of its biological roles, potentially severe eosinophilia, in AERD.¹⁰⁵

The role of hepatic cytochrome P450 enzymes in drug metabolism and response is well studied, and an abundance of pharmacogenetic studies has focused on the roles of these enzymes in response to various drug classes. Loss-of-function polymorphisms in *CYP2C19*, a major metabolizer of nonsteroidal anti-inflammatory drugs (NSAIDs) and AA metabolites, are more frequently expressed in Japanese patients with AERD and the percent predicted FEV₁ after lysine-aspirin challenge test in patients with the reference genotypes of *CYP2C19* 681G > A and 636G > A was higher than that seen in patients with GA/AA.¹⁰⁶ Because antileukotriene medications are more commonly prescribed for AERD, and the magnitude of treatment response to these medications among AERD patients is highly variable, discerning their routes of metabolism has relevance for therapeutic intervention, because all are either substrates and/or inhibitors of the highly polymorphic P450 enzymes. For example, montelukast is metabolized by *CYP2C8*,¹⁰⁷ zileuton is an inhibitor of *CYP1A2*¹⁰⁸ and a substrate of *CYP3A4*,¹⁰⁹ and zafirlukast is a substrate of *CYP2C9*.¹¹⁰ Although polymorphisms that predict altered clinical pharmacokinetics of diverse drug classes have been associated with these genes, to date, no pharmacogenomic studies have investigated potential associations of these genes with variation in therapeutic responses to antileukotrienes (or other asthma medications) in AERD patients.

Genetic Biomarkers and Predictive Tests

AERD diagnosis requires definitive confirmation by oral aspirin challenge, a time-consuming procedure during which severe clinical complications may occur. The potential of severe clinical complications arising due to provocation tests during diagnosis of AERD warrants the development of noninvasive diagnostic methods, such as biomarkers. One-fifth of severe asthmatics are unaware that they suffer from aspirin intolerance^{4,111} and may, therefore, be at risk of experiencing serious exacerbations during diagnosis and otherwise. Because AERD is often underdiagnosed due to poor patient and clinical awareness of symptoms, the ability to identify novel and more precise biomarkers (genetic, epigenetic, and proteomic) associated with specific clinical features and symptoms, as well as the endotype as a whole, can assist in efforts to better identify and appropriately treat at-risk individuals. This information can be used to develop a predictive diagnostic test that can avoid complications of aspirin administration in sensitive patients, avoiding exacerbations and need for increased dosages of asthma controller medication.

Data from genetic association studies are well-suited for the development of SNP-based tests for predicting clinical phenotypes. A study conducted in 2012 sought to use existing genotype data from 109,365 SNPs genotyped in the DNA samples of 100 AERD and 100 ATA subjects from a prior GWAS to develop a prognostic SNP test for AERD.¹¹² A set of 8 SNPs in 8 candidate genes had sufficient discriminative power to discern AERD versus ATA.¹¹² In addition to GWAS data, combining gene expression and proteomic data is also useful for identifying plasma-borne biomarkers to discriminate disease phenotypes. In an effort to develop diagnostic gene and protein biomarkers of AERD using microarray data from PBMCs, Shin and colleagues¹¹³ integrated mRNA expression profiles that were differentially expressed with regard to AERD versus ATA status with a database of secreted proteins, quantified the protein levels in plasma samples by ELISA, and assessed their discriminative ability for AERD versus ATA using ROC curve analysis. A total of 11 genes were identified as secreted proteins and validated by ELISA in patient plasma samples; among these, plasma levels of eosinophil-derived neurotoxin were significantly higher in AERD versus

ATA.¹¹³ Furthermore, plasma eosinophil-derived neurotoxin levels showed high sensitivity and high diagnostic accuracy for predicting AERD.¹¹³ The investigators propose that eosinophil-derived neurotoxin levels in plasma could serve as biomarker to distinguish AERD from ATA.

Limitations of These Studies

There is tremendous clinical value in developing a noninvasive, predictive diagnostic biomarker for AERD and its clinical phenotypes using data from genetic, pharmacogenetics, and biomarker studies; however, substantial challenges must first be overcome to accomplish this goal. A major challenge for biomarker studies is the availability of robust, noise-free input data (mRNAs, SNPs, and proteins) from the ideal physiologic compartments (plasma, serum, and so forth) that can best reflect the pathologic conditions of the disease state and also be readily sampled in a clinical setting. The predictive accuracy of individual biomarkers and SNPs is highly variable and greatly depends on the modeling approach used, sample size, and phenotypic variation within the sample measured. Relevant clinical covariates and comorbidities that could affect the variation in these biomarkers, such as medication use, gender, age, tissue/cell type, and disease severity, must also be accounted for in development of accurate predictive models. Validation of the predictive models in similar data sets and clinical samples is also needed to confirm that the models can reliably and accurately predict the phenotype.

SUMMARY

Although the molecular mechanisms that underlie AERD pathogenesis are not fully understood, genetic and epigenetic variation play a significant role. This review presents evidence from recent studies that point toward variation in diverse molecular pathways for arachidonic acid metabolism, T_H1 and T_H2 immune responses, inflammation, upper airway and nasal epithelial cell proliferation, eosinophilia, drug responses, and other pathways in AERD susceptibility and its unique clinical symptoms and response to therapy. The applications of whole-genome sequencing and next-generation technologies are anticipated to increase the likelihood of detecting potentially functional rare variants and increase the pool of associated loci. These and other association studies will require replication in diverse populations and must prioritize functional validation of new and existing associations. Epigenetic modification within B cells and NP epithelia in patients with AERD contribute an additional source of regulatory control for variation and gene expression in AERD severity. These studies are sparse, however, and also subject to the same challenges as genetic-association studies. Integration of whole-genome sequence, epigenetic, and gene expression data collected in studies with strong designs, for example, before and after ASA challenge or at disease inception, should be pursued. Finally, there is great promise in using well-validated genetic markers and proteins identified through these studies to develop predictive biomarkers that can lead to the development of noninvasive, diagnostic tests for AERD. An increased understanding of genetic and epigenetic mechanisms provides an opportunity to develop new therapeutic approaches for the diagnosis, treatment, and management of AERD.

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