Published in final edited form as:

Immunol Allergy Clin North Am. 2016 November ; 36(4): 765–789. doi:10.1016/j.iac.2016.06.010.

Genetic and Epigenetic Components of Aspirin-Exacerbated Respiratory Disease

Amber Dahlin, PhD, MMSc and **Scott T. Weiss, MD, MS***

Channing Division of Network Medicine, Brigham and Women's Hospital, Harvard Medical School, 181 Longwood Avenue, Boston, MA 02115, USA

Keywords

cysteinyl leukotriene; eosinophil; biomarker; epigenetics; polymorphism; AERD

I. INTRODUCTION

Aspirin intolerance is a severe and relatively 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, Aspirin Exacerbated Respiratory Disease (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, 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 following aspirin challenge and symptom exacerbations[5,6]. Due to the discovery that increased production of LTs is a characteristic of AERD, the leukotriene and prostaglandin 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 anti-leukotriene asthma medications contributed further evidence toward a mechanistic role for the 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 inter-individual treatment responses to multiple asthma drug classes including

*Corresponding author. Scott T. Weiss, 181 Longwood Ave #461, Boston, MA 02215, scott.weiss@hms.harvard.edu.

Disclosure Statement: The authors have nothing to disclose.

leukotriene modifiers and inhibitors[14]. However, the exact mechanisms by which LT synthesis becomes dysregulated in AERD are still unknown. Due to corresponding alteration of immune molecules (e.g. Th2 cytokines), prostaglandins (e.g. PGE_2), and other inflammatory biomarkers (e.g. IL-5, periostin, IgE, ApoA1 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 1–6% 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.

II. UPDATE ON THE GENETICS OF AERD

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 (Cys-LT) production, inflammatory cascades initiated by eosinophils, mast cells, platelets, airway epithelial cells, and others. For reference, we summarize the major results from these studies in Table 1. However, findings from many of these studies are conflicting, and the majority of reported associations lack replication. In this section, we provide a comprehensive update of the status of genetic investigations of aspirin-sensitive asthma and AERD, highlighting major discoveries published within the last several years. In addition to discussing genetic association studies of AERD risk, we will also present recent findings from investigations of genetic markers associated with two predominant AERD clinical featuresnasal polyposis[2] and eosiniophilia[6].

A. Genetic markers associated with disease status and clinical features of AERD

1. AERD 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). In this section we will discuss recent discoveries with compelling evidence for a role in AERD pathogenesis.

The best mechanistic evidence for AERD pathogenesis supports intrinsic dysregulation of the activity of the 5-LO/LTC_4S 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 are 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 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 aspirin-tolerant asthmatic (ATA) populations carrying the CC or AC genotype vs. AA genotype (OR = 1.36, 95% CI = 1.12–1.65, $p = 0.002$) but not in aspirin-intolerant groups (OR = 1.16, 95% CI = 0.89–1.52, $p = 0.27$)[31]. Therefore, variation in *LTC4S*, while 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] (Table 1). However, new *ALOX5* variants associated with AERD have not been identified. The *CYSLTR1* and *CYSLTR2* leukotriene receptor genes are among the most important for leukotriene signaling, and are pharmacological targets for montelukast, the 'gold standard' prescribed medication for AERD symptom control[34–36]. *CYSLTR1* is over-expressed in nasal tissues of AERD patients, and three promoter SNPs in *CYSLTR1* have been associated with both AERD status and higher *CYSLTR1* promoter activity, suggesting that functional variation driving over-expression of this receptor underlies its pathological roles in LT signaling in AERD[37,38] (Table 1). Polymorphisms in *CYSLTR2* are also associated with AERD and FEV1 decline following aspirin provocation test (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 non-functional 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 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-HETE, an arachidonic acid pathway metabolite that is released at higher levels in eosinophils from severely affected AERD patients[41]. While 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 were found[42]. Clearly 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.

2. Genetic associations with nasal polyposis in AERD/AIA—AERD comprises up to 30% of asthmatics with nasal polyps (NPs)[25,43]. Inflammatory mediators in the Th2 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 post-surgical symptomatic recurrence, than patients with non-AERD related chronic rhinosinusitis with nasal polyps[44–46]. However, the genetic and molecular mechanisms that

can differentiate this particular AERD phenotype from non-AERD phenotypes with nasal polyposis are unclear. Comparison of an inflammatory response signature including Th2 and non-Th2 cytokine and chemokine encoding genes, identified from microarray expression profiling of inflammatory mediators within nasal polyp samples from patients with chronic rhinosinusitis vs. patients with AERD, revealed significantly elevated expression of five mediators (eosinophilic cationic protein (*ECP*), *GM-CSF, SDF-1* and *SDF1, MCP-1* and *IL10*), and reduced expression of *tissue plasminogen activator* (*TPA*), in the nasal polyps of AERD[47]. AERD nasal polyps also contained significantly elevated protein levels of ECP, GM-CSF and MCP-1 as compared to the chronic rhinosinusitis samples[47], as well as increased eosinophilia. However, no corresponding increase in Th2-specific protein expression was associated with eosinophil proliferation and recruitment in AERD samples, suggesting that other, non-Th2 processes, may be important for AERD pathogenesis[47].

An MHC related gene, *class II major histocompatibility complex transactivator (CIITA)*, is expressed in nasal polyps, 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] SNPs hypothesized to play a role in AERD were genotyped in 158 AERD patients and 309 ATA of Korean ancestry, and one SNP, rs1139564, was nominally associated with nasal polyps in the AERD group[48]. However, this association did not persist after multiple test correction[48].

As eicosanoids and their receptors are upregulated in inflammatory cells within nasal polyp tissue[49,50], corresponding to high levels of *LTC4S*[51], *CYSLTR1*[20] and *PTGDR*[25] transcript expression in nasal polyps, 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=0.009) and in patients with the aspirin triad (OR 0.25; 95% CI 0.09–0.72; p=0.005)[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=0.04)[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 nasal polyps[54], pointing toward an important role for this gene in development of this phenotype.

In summary, genetic studies of nasal polyposis in AERD/AIA implicate inflammation and the eicosanoid pathway.

3. Genetic associations with eosinophilia in AERD/AIA—Persistent eosinophilia and cytokine over-production are critical clinical features of AERD[47]; furthermore, eosinophil activation and migration require the presence of cytokines and other immune mediators. Eosinophilia is a Th2-cytokine-dependent process, and expression of IL-4, IL-5,

IL-13 and other cytokines correlate with eosinophilic infiltration[43,47]. Th2 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 three 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 authors 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 NF-KB 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*, *−50C>T*) were significantly associated with the risk of aspirin intolerance as well as the rate of decline in FEV_1 following aspirin challenge, although the minor allele frequencies for all three SNPs were significantly lower for AERD[59]. Finally, *IL17RA* expression in $CD14^+$ monocytes from asthmatic patients with all three minor allele genotypes for *IL17RA* −1075A>G, −947A>G, −50C>T was significantly higher than for the reference homozygotes[59]. The minor alleles of the three 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 following 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 while *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 hyperreactivity 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 et al. profiled the expression of Th1 and Th2 cytokines in tissue samples from 30 asthmatics with chronic hyperplastic eosinophilic sinusitis, 15 patients with AERD, and 9 healthy controls, using quantitative real-time PCR (RT-PCR) (Steinke)[64]. The authors determined that while a Th2 cytokine signature, as shown by increased expression of *IL-4, IL-5*, and *IL-13* as compared with control tissue, dominated in both AERD and ATA, AERD alone demonstrated over-expression of Th1 cytokine IFN-γ which was also determined as originating from eosinophils through flow cytometry and histological studies [64]. Furthermore, in addition to priming these eosinophils, IFN-γ also 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 following 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, 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 minor allele frequencies 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 following 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 heat shock protein family members, in particular HSP70, are correlated with asthma severity and eosinophilia and overexpressed in the asthmatic airway[69,70]. However, the role of HSPs in allergic asthma is unclear. To investigate the hypothesis that HSP70 variation contributes to AERD susceptibility, a recent study evaluated the association of two candidate HSP70 polymorphisms- *HSPA1B*-179C>T and *HSPA1B*-1267A>G (rs6457452 and rs1061581) in 102 asthmatics with AERD, 300 ATA, and 100 normal healthy controls, all of Japanese descent[71]. In patients with AERD, compared to the aspirin tolerant 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 vs. ATA 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]. While the molecular mechanisms of HSP70 variation and eosinophilia in AERD were not investigated in this study, the authors 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].

B. Genome-wide approaches for investigating genetic relationships in AERD

To date, multiple genetic risk factors for AERD have been identified through candidate gene studies and GWAS (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 et al. profiled 275,862 SNPs from 179 AERD patients, 211 patients with aspirin exacerbated cutaneous disease (AECD), and 1989 healthy control subjects[73]. While 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 hyperresponsiveness 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 Illumina Human660W BeadChip, in 117 subjects with AERD and 685 ATA patients[7]. 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 following 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.

While 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 genome 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 utilizing microarray expression profiling and a candidate gene analysis in PBMCs from a small Caucasian population identified three genes with expression profiles that significantly differed between AIA *vs.* ATA and/or AIA *vs.* healthy subjects[75]. In particular, expression of *CNPY3* and *FOSL1* were significantly lower in AIA vs healthy controls, while *ERAS* expression was increased. Protein expression of *FOSL1* in PBMCs was also significantly lower for AIA than the control groups[75]. While

the study lacked mechanistic investigation of these novel candidate genes for AERD, the authors suggest these genes could participate in innate immune response pathways and pathways for tissue/cell remodeling and airway hyperresponsiveness 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 et al. recently used an exome-wide profiling approach using the HumanExome BeadChip v1.1 (Illumina Inc.) 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[76]. After filtering and quality control of genotype data, over 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 GWAS[77] had identified exm537522 (also annotated as rs1042151 in that study) as having the strongest association with AERD susceptibility; therefore, the authors replicated one of their top associations from a previous study[76]. To develop a predictive model for AERD risk, the authors selected the best combination of the top 10 SNPs that could discriminate between AERD and ATA, using multiple logistic regression, and calculated ROC curves and 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 vs. 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 studies tend to support the involvement of the HLA locus in the pathogenesis of AERD.

C. Limitations of these studies

A significant limitation of genetic studies of AERD is that very 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, the 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.

III. EPIGENETICS OF AERD

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. Further, 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, we will discuss insights from recent studies of epigenetic modifications in AERD vs. other allergic asthma endotypes.

A. Global investigations of epigenetic modifications in AERD

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 in the previous section, nasal polyps 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 nasal polyp samples from five patients with AIA and four 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 to the patients with ATA, while 158 sites in 141 genes were significantly decreased, while buffy coat DNA methylation patterns were not significantly diverse between the two 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 polyposis. In particular, methylation patterns for four genes (*PGDS*, *ALOX5AP*, *PTGES* and *LTB4)* that drive the arachidonic acid metabolism pathway that is uniquely dysregulated in AERD, were altered; *PGDS*, *ALOX5AP* were hypomethylated, whereas *PTGES* was hypermethylated, suggesting that altered methylation patterns regulating expression of these genes could underlie aspirin hypersensitivity[79]. In addition, two Th2 cytokine encoding genes, *IL5RA* and *IL10*, were also 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].

B. Functional epigenetics of AERD

The role of epigenetic targeting of PGE^2 pathway genes involved in the expansion of nasal polyps 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 upregulated in AERD, and stimulation of the EP_2 receptor by PGE_2 represses the activation and growth of these cells. Prior evidence from an epigenome-wide association study revealed that *PTGES*, the gene encoding a microsomal PGE synthase (mPGES-1) that converts PGH_2 to PGE_2, was hypermethylated in nasal polyp tissue from AERD subjects[79]. In addition, fibroblasts from nasal polyps of patients with AERD have intrinsically lower expression of COX-2, PGE_2 and EP_2 receptor protein vs. aspirin-tolerant (AT) control subjects[81]. Cahill et al. hypothesized that an intrinsic defect in EP_2 expression in nasal polyp fibroblasts, potentially a result of epigenetic modification at this locus, underlies the aggressive expansion and proliferation of nasal polyps in AERD patients[80]. To investigate this, the authors first isolated and cultured fibroblasts from nasal polyps of 18 patients with AERD and nine aspirin-tolerant patients with chronic rhinosinusitis and nasal polyposis, and nasal tissue from eight non-asthmatic 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 PGE_2, as well as having reduced expression levels of the EP_2 receptor[80]. In addition, in AERD samples, EP_2 receptor mRNA was significantly up-regulated by treatment of the fibroblasts with the histone deacetylase inhibitor TSA, and histone acetylation (H3K27ac) at the EP_2 promoter correlated strongly with baseline EP_2 mRNA expression levels[80]. However, DNA methylation at the EP_2 promoter in fibroblasts was not significantly different, suggesting that histone modification was more likely to contribute to EP_2 expression in nasal fibroblasts in AERD[80]. Together, these findings support a role of epigenetic effects in AERD.

C. 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 in order 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.

IV. IMPACT OF GENETICS IN THE CLINICAL MANAGEMENT OF AERD

LTs are bioactive lipids derived from arachidonic acid (AA) that serve as immunological 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 forced expiratory volume in 1 second (FEV_1), compared to non-AERD asthmatics, and significantly higher baseline and post-aspirin levels of urinary LTE_4, 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, positively impacting 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 nasal polyp recurrence are achieved with aspirin desensitization and daily high-dose aspirin treatment[90]. The dramatic increase in LT production immediately following 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 over-production leading to a pro-inflammatory state to inform understanding of LT biology and treatment response.

A. Pharmacogenetics

A number of pharmacogenetic studies of treatment responses in AIA have been performed. We will discuss recent investigations in this section. Due to its ability to inhibit LT-mediated airway inflammation by blocking CysLT1 receptors, montelukast treatment might ideally benefit specific asthmatic patient subgroups with over-production of LTs as a clinical feature, including patients with aspirin hypersensitivity[93]. Montelukast as mono- 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 β2 agonists[93]. Candidate gene and genome-wide investigations of anti-LT responses implicate involvement of multiple genes, including *ALOX5*[32], *ALOX5AP*[34,35,94–96], *LTC4S30*,[34–36,87,97], *CYSLTR1*[34–36,87], *CYSLTR2*[34,97], *ABCC1*[35,36,97–99] and *OATP2B1*[100]. Recently, we conducted the first pharmacogenomics GWAS studies of zileuton[101] and montelukast[102] 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 anti-leukotrienes[105]. A total of 122 patients with AERD were classified according to symptomatic response to aspirin rechallenge following one or more years of treatment with asthma controller medications; group I patients (N=48) negative conversions to follow-up lysine-aspirin bronchoprovocation test (L-ASA BPT) while group II patients (N=74) 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>1, *ALOX15* 427G>A, *CCR3* 520T>G, *CRTH2* 466T>G, *CYSLTR1* 634C>T, *IL10* 1082A>G, *IL13* 1055C>T, *LTC4S* 444A>C, *TGFb* 509C>T, *TNFa* 308G>A and *HLADPB1**0301) was conducted[105]. There were no significant differences in genotype frequencies between the two 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 following L-ASA BPT[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 upregulated in nasal polyp 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 have focused on the roles of these enzymes in response to various drug classes. Loss-of-function polymorphisms in *CYP2C19*, a major metabolizer of NSAIDs and AA metabolites, are more frequently expressed in Japanese patients with AERD and the percent predicted FEV_1 following lysine-aspirin challenge test in patents with the reference genotypes of *CYP2C19* 681G>A and 636G>A was higher than that seen in patients with GA/AA[106]. Because anti-leukotriene 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, as 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 substrate of CYP3A4[109], and zafirlukast is a substrate of CYP2C9[110]. While 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 anti-leukotrienes (or other asthma medications) in AERD patients.

B. 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 non-invasive diagnostic methods such as biomarkers. Of note, 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. As AERD is often under-diagnosed 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 utilize 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 eight SNPs in eight candidate genes had sufficient discriminative power to discern AERD vs. 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 et al. integrated mRNA expression profiles that were differentially expressed with regard to AERD vs. ATA status with a database of secreted proteins, quantified the protein levels in plasma samples by enzyme-linked immunosorbent assay (ELISA), and assessed their discriminative ability for AERD vs. ATA using ROC curve analysis[113]. 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 vs. ATA[113]. Furthermore, plasma eosinophil-derived neurotoxin levels showed high sensitivity and high diagnostic accuracy for predicting AERD[113]. The authors propose that eosinophil-derived neurotoxin levels in plasma could serve as biomarker to distinguish AERD from ATA.

C. Limitations of these studies

There is tremendous clinical value in developing a non-invasive, predictive diagnostic biomarker for AERD and its clinical phenotypes using data from genetic, pharmacogenetic 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 physiological compartments (plasma, serum, etc.) that can best reflect the pathological 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 upon 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 in order to confirm that the models can reliably and accurately predict the phenotype.

V. SUMMARY

While the molecular mechanisms that underlie AERD pathogenesis are not fully understood, genetic and epigenetic variation plays a significant role. In this review, we presented evidence from recent studies that point toward variation in diverse molecular pathways for arachidonic acid metabolism, Th1 and Th2 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 application 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. However, 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 nasal polyp epithelia in patients with AERD contribute an additional source of regulatory control for variation and gene expression in AERD severity. However, these studies are sparse 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 e.g. 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 non-invasive, 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.

Acknowledgments

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).

References

1. Jenkins C, Costello J, Hodge L. Systematic review of prevalence of aspirin induced asthma and its implications for clinical practice. BMJ. 2004; 328(7437):434. [PubMed: 14976098]
2. Vally H, Taylor ML, Thompson PJ. The prevalence of aspirin intolerant asthma (AIA) in Australian asthmatic patients. Thorax. 2002; 57(7):569–574. [PubMed: 12096197]
3. Kasper L, Sladek K, Duplaga M, et al. Prevalence of asthma with aspirin hypersensitivity in the adult population of Poland. Allergy. 2003; 58(10):1064–1066. [PubMed: 14510727]
4. Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. Eur Respir J. 2000; 16(3):432–436. [PubMed: 11028656]

5. Stevenson DD. Aspirin sensitivity and desensitization for asthma and sinusitis. Curr Allergy Asthma Rep. 2009; 9(2):155–163. [PubMed: 19210906]
6. Choi JH, Kim MA, Park HS. An update on the pathogenesis of the upper airways in aspirin-exacerbated respiratory disease. Curr Opin Allergy Clin Immunol. 2014; 14(1):1–6. [PubMed: 24300420]
7. Palikhe NS, Kim JH, Park HS. Update on recent advances in the management of aspirin exacerbated respiratory disease. Yonsei Med J. 2009; 50(6):744–750. [PubMed: 20046412]
8. Kim SH, Sanak M, Park HS. Genetics of hypersensitivity to aspirin and nonsteroidal anti-inflammatory drugs. Immunol Allergy Clin North Am. 2013; 33(2):177–194. [PubMed: 23639707]
9. Busse WW, McGill KA, Horwitz RJ. Leukotriene pathway inhibitors in asthma and chronic obstructive pulmonary disease. Clin Exp Allergy. 1999; 29(Suppl 2):110–115. [PubMed: 10421833]
10. Park HS. Aspirin-sensitive asthma: recent advances in management. BioDrugs. 2000; 13(1):29–33. [PubMed: 18034511]
11. Nathan RA, Kemp JP, Group AW. Efficacy of antileukotriene agents in asthma management. Ann Allergy Asthma Immunol. 2001; 86(6 Suppl 1):9–17. [PubMed: 11426917]
12. Berges-Gimeno MP, Simon RA, Stevenson DD. The effect of leukotriene-modifier drugs on aspirin-induced asthma and rhinitis reactions. Clin Exp Allergy. 2002; 32(10):1491–1496. [PubMed: 12372130]
13. Dahlén SE. Lipid mediator pathways in the lung: leukotrienes as a new target for the treatment of asthma. Clin Exp Allergy. 1998; 28(Suppl 5):141–146. discussion 171–143.
14. Ind PW. Anti-leukotriene intervention: is there adequate information for clinical use in asthma? Respir Med. 1996; 90(10):575–586. [PubMed: 8959114]
15. Berges-Gimeno MP, Simon RA, Stevenson DD. The natural history and clinical characteristics of aspirin-exacerbated respiratory disease. Ann Allergy Asthma Immunol. 2002; 89(5):474–478. [PubMed: 12452205]
16. Cowburn AS, Sladek K, Soja J, et al. Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma. J Clin Invest. 1998; 101(4):834–846. [PubMed: 9466979]
17. Sampson AP, Cowburn AS, Sladek K, et al. Profound overexpression of leukotriene C4 synthase in bronchial biopsies from aspirin-intolerant asthmatic patients. Int Arch Allergy Immunol. 1997; 113(1–3):355–357. [PubMed: 9130576]
18. Kim SH, Choi JH, Holloway JW, et al. Leukotriene-related gene polymorphisms in patients with aspirin-intolerant urticaria and aspirin-intolerant asthma: differing contributions of ALOX5 polymorphism in Korean population. J Korean Med Sci. 2005; 20(6):926–931. [PubMed: 16361798]
19. Kim SH, Bae JS, Suh CH, Nahm DH, Holloway JW, Park HS. Polymorphism of tandem repeat in promoter of 5-lipoxygenase in ASA-intolerant asthma: a positive association with airway hyperresponsiveness. Allergy. 2005; 60(6):760–765. [PubMed: 15876305]
20. Sousa AR, Parikh A, Scadding G, Corrigan CJ, Lee TH. Leukotriene-receptor expression on nasal mucosal inflammatory cells in aspirin-sensitive rhinosinusitis. N Engl J Med. 2002; 347(19):1493–1499. [PubMed: 12421891]
21. Arm JP, O'Hickey SP, Spur BW, Lee TH. Airway responsiveness to histamine and leukotriene E4 in subjects with aspirin-induced asthma. Am Rev Respir Dis. 1989; 140(1):148–153. [PubMed: 2546469]
22. Kim SH, Oh JM, Kim YS, et al. Cysteinyl leukotriene receptor 1 promoter polymorphism is associated with aspirin-intolerant asthma in males. Clin Exp Allergy. 2006; 36(4):433–439. [PubMed: 16630147]
23. Akahoshi M, Obara K, Hirota T, et al. Functional promoter polymorphism in the TBX21 gene associated with aspirin-induced asthma. Hum Genet. 2005; 117(1):16–26. [PubMed: 15806396]
24. Ying S, Meng Q, Scadding G, Parikh A, Corrigan CJ, Lee TH. Aspirin-sensitive rhinosinusitis is associated with reduced E-prostanoid 2 receptor expression on nasal mucosal inflammatory cells. J Allergy Clin Immunol. 2006; 117(2):312–318. [PubMed: 16461132]

25. Pérez-Novo CA, Watelet JB, Claeys C, Van Cauwenberge P, Bachert C. Prostaglandin, leukotriene, and lipoxin balance in chronic rhinosinusitis with and without nasal polyposis. J Allergy Clin Immunol. 2005; 115(6):1189–1196. [PubMed: 15940133]
26. Schmid M, Göde U, Schäfer D, Wigand ME. Arachidonic acid metabolism in nasal tissue and peripheral blood cells in aspirin intolerant asthmatics. Acta Otolaryngol. 1999; 119(2):277–280. [PubMed: 10320091]
27. Wang G, Zhang J, Sun H, Cao W, Wang Y, Xiao H. Genetic variation in members of the leukotrienes biosynthesis pathway confers risk of ischemic stroke in Eastern Han Chinese. Prostaglandins Leukot Essent Fatty Acids. 2012; 87(6):169–175. [PubMed: 23079278]
28. Wang GN, Zhang JS, Cao WJ, et al. Association of ALOX5, LTA4H and LTC4S gene polymorphisms with ischemic stroke risk in a cohort of Chinese in east China. World J Emerg Med. 2013; 4(1):32–37. [PubMed: 25215090]
29. Zhao N, Liu X, Wang Y, et al. Association of inflammatory gene polymorphisms with ischemic stroke in a Chinese Han population. J Neuroinflammation. 2012; 9:162. [PubMed: 22769019]
30. Lima JJ, Zhang S, Grant A, et al. Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. Am J Respir Crit Care Med. 2006; 173(4):379–385. [PubMed: 16293801]
31. Zhang Y, Huang H, Huang J, et al. The −444A/C polymorphism in the LTC4S gene and the risk of asthma: a meta-analysis. Arch Med Res. 2012; 43(6):444–450. [PubMed: 22884858]
32. Drazen JM, Yandava CN, Dubé L, et al. Pharmacogenetic association between ALOX5 promoter genotype and the response to anti-asthma treatment. Nat Genet. 1999; 22(2):168–170. [PubMed: 10369259]
33. Kim SH, Park HS. Genetic markers for differentiating aspirin-hypersensitivity. Yonsei Med J. 2006; 47(1):15–21. [PubMed: 16502481]
34. Klotsman M, York TP, Pillai SG, et al. Pharmacogenetics of the 5-lipoxygenase biosynthetic pathway and variable clinical response to montelukast. Pharmacogenet Genomics. 2007; 17(3): 189–196. [PubMed: 17460547]
35. Lima JJ. Treatment heterogeneity in asthma: genetics of response to leukotriene modifiers. Mol Diagn Ther. 2007; 11(2):97–104. [PubMed: 17397245]
36. Tantisira KG, Lima J, Sylvia J, Klanderman B, Weiss ST. 5-lipoxygenase pharmacogenetics in asthma: overlap with Cys-leukotriene receptor antagonist loci. Pharmacogenet Genomics. 2009; 19(3):244–247. [PubMed: 19214143]
37. Laidlaw TM, Boyce JA. Pathogenesis of aspirin-exacerbated respiratory disease and reactions. Immunol Allergy Clin North Am. 2013; 33(2):195–210. [PubMed: 23639708]
38. Laidlaw TM, Boyce JA. Platelets in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol. 2015; 135(6):1407–1414. quiz 1415. [PubMed: 26051947]
39. Poon AH, Houseman EA, Ryan L, Sparrow D, Vokonas PS, Litonjua AA. Variants of asthma and chronic obstructive pulmonary disease genes and lung function decline in aging. J Gerontol A Biol Sci Med Sci. 2014; 69(7):907–913. [PubMed: 24253534]
40. Allen M, Heinzmann A, Noguchi E, et al. Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nature genetics. 2003; 35(3):258–263. [PubMed: 14566338]
41. Kim SH, Choi H, Yoon MG, Ye YM, Park HS. Dipeptidyl-peptidase 10 as a genetic biomarker for the aspirin-exacerbated respiratory disease phenotype. Ann Allergy Asthma Immunol. 2015; 114(3):208–213. [PubMed: 25592153]
42. Chang HS, Park JS, Shin HR, Park BL, Shin HD, Park CS. Association analysis of FABP1 gene polymorphisms with aspirin-exacerbated respiratory disease in asthma. Exp Lung Res. 2014; 40(10):485–494. [PubMed: 25338211]
43. Steinke JW, Borish L. Factors driving the aspirin exacerbated respiratory disease phenotype. Am J Rhinol Allergy. 2015; 29(1):35–40. [PubMed: 25590316]
44. Kim JE, Kountakis SE. The prevalence of Samter's triad in patients undergoing functional endoscopic sinus surgery. Ear Nose Throat J. 2007; 86(7):396–399. [PubMed: 17702319]
45. Robinson JL, Griest S, James KE, Smith TL. Impact of aspirin intolerance on outcomes of sinus surgery. Laryngoscope. 2007; 117(5):825–830. [PubMed: 17473677]

46. Awad OG, Lee JH, Fasano MB, Graham SM. Sinonasal outcomes after endoscopic sinus surgery in asthmatic patients with nasal polyps: a difference between aspirin-tolerant and aspirin-induced asthma? Laryngoscope. 2008; 118(7):1282–1286. [PubMed: 18475212]
47. Stevens WW, Ocampo CJ, Berdnikovs S, et al. Cytokines in Chronic Rhinosinusitis. Role in Eosinophilia and Aspirin-exacerbated Respiratory Disease. Am J Respir Crit Care Med. 2015; 192(6):682–694. [PubMed: 26067893]
48. Bae JS, Pasaje CF, Park BL, et al. Genetic association analysis of CIITA variations with nasal polyp pathogenesis in asthmatic patients. Mol Med Rep. 2013; 7(3):927–934. [PubMed: 23292525]
49. Baenkler HW, Schäfer D, Hosemann W. Eicosanoids from biopsy of normal and polypous nasal mucosa. Rhinology. 1996; 34(3):166–170. [PubMed: 8938887]
50. Yoshimura T, Yoshikawa M, Otori N, Haruna S, Moriyama H. Correlation between the prostaglandin D(2)/E(2) ratio in nasal polyps and the recalcitrant pathophysiology of chronic rhinosinusitis associated with bronchial asthma. Allergol Int. 2008; 57(4):429–436. [PubMed: 18797183]
51. Adamjee J, Suh YJ, Park HS, et al. Expression of 5-lipoxygenase and cyclooxygenase pathway enzymes in nasal polyps of patients with aspirin-intolerant asthma. J Pathol. 2006; 209(3):392–399. [PubMed: 16583357]
52. Benito Pescador D, Isidoro-García M, García-Solaesa V, et al. Genetic association study in nasal polyposis. J Investig Allergol Clin Immunol. 2012; 22(5):331–340.
53. Batra J, Pratap Singh T, Mabalirajan U, Sinha A, Prasad R, Ghosh B. Association of inducible nitric oxide synthase with asthma severity, total serum immunoglobulin E and blood eosinophil levels. Thorax. 2007; 62(1):16–22. [PubMed: 17189532]
54. Pascual M, Suzuki M, Isidoro-Garcia M, et al. Epigenetic changes in B lymphocytes associated with house dust mite allergic asthma. Epigenetics. 2011; 6(9):1131–1137. [PubMed: 21975512]
55. Losol P, Kim SH, Shin YS, Ye YM, Park HS. A genetic effect of IL-5 receptor α polymorphism in patients with aspirin-exacerbated respiratory disease. Exp Mol Med. 2013; 45:e14. [PubMed: 23470716]
56. Louten J, Boniface K, de Waal Malefyt R. Development and function of TH17 cells in health and disease. J Allergy Clin Immunol. 2009; 123(5):1004–1011. [PubMed: 19410689]
57. Kawaguchi M, Adachi M, Oda N, Kokubu F, Huang SK. IL-17 cytokine family. J Allergy Clin Immunol. 2004; 114(6):1265–1273. quiz 1274. [PubMed: 15577820]
58. Jung JS, Park BL, Cheong HS, et al. Association of IL-17RB gene polymorphism with asthma. Chest. 2009; 135(5):1173–1180. [PubMed: 19118269]
59. Park JS, Park BL, Kim MO, et al. Association of single nucleotide polymorphisms on Interleukin 17 receptor A (IL17RA) gene with aspirin hypersensitivity in asthmatics. Hum Immunol. 2013; 74(5):598–606. [PubMed: 23220496]
60. Dekker JW, Nizankowska E, Schmitz-Schumann M, et al. Aspirin-induced asthma and HLA-DRB1 and HLA-DPB1 genotypes. Clin Exp Allergy. 1997; 27(5):574–577. [PubMed: 9179433]
61. Choi JH, Lee KW, Oh HB, et al. HLA association in aspirin-intolerant asthma: DPB1*0301 as a strong marker in a Korean population. J Allergy Clin Immunol. 2004; 113(3):562–564. [PubMed: 15007363]
62. Park HS, Kim SH, Sampson AP, Lee KW, Park CS. The HLA-DPB1*0301 marker might predict the requirement for leukotriene receptor antagonist in patients with aspirin-intolerant asthma. J Allergy Clin Immunol. 2004; 114(3):688–689. [PubMed: 15446291]
63. Esmaeilzadeh H, Nabavi M, Amirzargar AA, et al. HLA-DRB and HLA-DQ genetic variability in patients with aspirin-exacerbated respiratory disease. Am J Rhinol Allergy. 2015; 29(3):e63–69. [PubMed: 25975240]
64. Steinke JW, Liu L, Huyett P, Negri J, Payne SC, Borish L. Prominent role of IFN-γ in patients with aspirin-exacerbated respiratory disease. J Allergy Clin Immunol. 2013; 132(4):856–865. e851–853. [PubMed: 23806637]
65. Early SB, Barekzi E, Negri J, Hise K, Borish L, Steinke JW. Concordant modulation of cysteinyl leukotriene receptor expression by IL-4 and IFN-gamma on peripheral immune cells. Am J Respir Cell Mol Biol. 2007; 36(6):715–720. [PubMed: 17272825]

66. Park JS, Heo JS, Chang HS, et al. Association analysis of member RAS oncogene family gene polymorphisms with aspirin intolerance in asthmatic patients. DNA Cell Biol. 2014; 33(3):155–161. [PubMed: 24555545]

67. Ferro E, Goitre L, Retta SF, Trabalzini L. The Interplay between ROS and Ras GTPases: Physiological and Pathological Implications. J Signal Transduct. 2012; 2012:365769. [PubMed: 22175014]

68. Takai Y, Sasaki T, Matozaki T. Small GTP-binding proteins. Physiol Rev. 2001; 81(1):153–208. [PubMed: 11152757]

69. Bertorelli G, Bocchino V, Zhuo X, et al. Heat shock protein 70 upregulation is related to HLA-DR expression in bronchial asthma. Effects of inhaled glucocorticoids. Clin Exp Allergy. 1998; 28(5): 551–560. [PubMed: 9645591]

70. Vignola AM, Chanez P, Polla BS, Vic P, Godard P, Bousquet J. Increased expression of heat shock protein 70 on airway cells in asthma and chronic bronchitis. Am J Respir Cell Mol Biol. 1995; 13(6):683–691. [PubMed: 7576706]

71. Kikuchi K, Abe S, Kodaira K, et al. Heat shock protein 70 gene polymorphisms in Japanese patients with aspirin-exacerbated respiratory disease. J Investig Med. 2013; 61(4):708–714.

72. Milner CM, Campbell RD. Structure and expression of the three MHC-linked HSP70 genes. Immunogenetics. 1990; 32(4):242–251. [PubMed: 1700760]

73. Kim SH, Cho BY, Choi H, et al. The SNP rs3128965 of HLA-DPB1 as a genetic marker of the AERD phenotype. PLoS One. 2014; 9(12):e111220. [PubMed: 25536158]

74. Park BL, Kim TH, Kim JH, et al. Genome-wide association study of aspirin-exacerbated respiratory disease in a Korean population. Hum Genet. 2013; 132(3):313–321. [PubMed: 23180272]

75. Wieczfinska J, Kacprzak D, Pospiech K, et al. The whole-genome expression analysis of peripheral blood mononuclear cells from aspirin sensitive asthmatics versus aspirin tolerant patients and healthy donors after in vitro aspirin challenge. Respir Res. 2015; 16(1):147. [PubMed: 26646719]

76. Shin SW, Park BL, Chang H, et al. Exonic variants associated with development of aspirin exacerbated respiratory diseases. PLoS One. 2014; 9(11):e111887. [PubMed: 25372592]

77. Kim JH, Park BL, Cheong HS, et al. Genome-wide and follow-up studies identify CEP68 gene variants associated with risk of aspirin-intolerant asthma. PLoS One. 2010; 5(11):e13818. [PubMed: 21072201]

78. Barker DJ. The origins of the developmental origins theory. J Intern Med. 2007; 261(5):412–417. [PubMed: 17444880]

79. Cheong HS, Park SM, Kim MO, et al. Genome-wide methylation profile of nasal polyps: relation to aspirin hypersensitivity in asthmatics. Allergy. 2011; 66(5):637–644. [PubMed: 21121930]

80. Cahill KN, Raby BA, Zhou X, et al. Impaired E Prostanoid2 Expression and Resistance to Prostaglandin E2 in Nasal Polyp Fibroblasts from Subjects with Aspirin-Exacerbated Respiratory Disease. Am J Respir Cell Mol Biol. 2016; 54(1):34–40. [PubMed: 26051534]

81. Roca-Ferrer J, Garcia-Garcia FJ, Pereda J, et al. Reduced expression of COXs and production of prostaglandin E(2) in patients with nasal polyps with or without aspirin-intolerant asthma. J Allergy Clin Immunol. 2011; 128(1):66–72. e61. [PubMed: 21397936]

82. Hammarström S. Biosynthesis and metabolism of leukotrienes. Monogr Allergy. 1983; 18:265–271. [PubMed: 6316129]

83. Hammarström S. Leukotrienes. Annu Rev Biochem. 1983; 52:355–377. [PubMed: 6311078]

84. Osher E, Weisinger G, Limor R, Tordjman K, Stern N. The 5 lipoxygenase system in the vasculature: emerging role in health and disease. Mol Cell Endocrinol. 2006; 252(1–2):201–206. [PubMed: 16647809]

85. Salmon JA, Higgs GA. Prostaglandins and leukotrienes as inflammatory mediators. Br Med Bull. 1987; 43(2):285–296. [PubMed: 2825898]

86. Sharma JN, Mohammed LA. The role of leukotrienes in the pathophysiology of inflammatory disorders: is there a case for revisiting leukotrienes as therapeutic targets? Inflammopharmacology. 2006; 14(1–2):10–16. [PubMed: 16835707]

87. Duroudier NP, Tulah AS, Sayers I. Leukotriene pathway genetics and pharmacogenetics in allergy. Allergy. 2009; 64(6):823–839. [PubMed: 19416143]

88. Israel E, Fischer AR, Rosenberg MA, et al. The pivotal role of 5-lipoxygenase products in the reaction of aspirin-sensitive asthmatics to aspirin. Am Rev Respir Dis. 1993; 148(6 Pt 1):1447–1451. [PubMed: 8256883]

89. Christie PE, Tagari P, Ford-Hutchinson AW, et al. Urinary leukotriene E4 concentrations increase after aspirin challenge in aspirin-sensitive asthmatic subjects. Am Rev Respir Dis. 1991; 143(5 Pt 1):1025–1029. [PubMed: 1850964]

90. Lee RU, Stevenson DD. Aspirin-exacerbated respiratory disease: evaluation and management. Allergy Asthma Immunol Res. 2011; 3(1):3–10. [PubMed: 21217919]

91. McDonald JR, Mathison DA, Stevenson DD. Aspirin intolerance in asthma. Detection by oral challenge. J Allergy Clin Immunol. 1972; 50(4):198–207. [PubMed: 5073322]

92. Delaney JC. The diagnosis of aspirin idiosyncrasy by analgesic challenge. Clin Allergy. 1976; 6(2): 177–181. [PubMed: 1277441]

93. Pacheco Y, Hosni R, Chabannes B, et al. Leukotriene B4 level in stimulated blood neutrophils and alveolar macrophages from healthy and asthmatic subjects. Effect of beta-2 agonist therapy. Eur J Clin Invest. 1992; 22(11):732–739. [PubMed: 1335872]

94. Holloway JW, Barton SJ, Holgate ST, Rose-Zerilli MJ, Sayers I. The role of LTA4H and ALOX5AP polymorphism in asthma and allergy susceptibility. Allergy. 2008; 63(8):1046–1053. [PubMed: 18547289]

95. Tcheurekdjian H, Via M, De Giacomo A, et al. ALOX5AP and LTA4H polymorphisms modify augmentation of bronchodilator responsiveness by leukotriene modifiers in Latinos. J Allergy Clin Immunol. 2010; 126(4):853–858. [PubMed: 20810156]

96. Via M, De Giacomo A, Corvol H, et al. The role of LTA4H and ALOX5AP genes in the risk for asthma in Latinos. Clin Exp Allergy. 2010; 40(4):582–589. [PubMed: 20067482]

97. Lima JJ, Blake KV, Tantisira KG, Weiss ST. Pharmacogenetics of asthma. Curr Opin Pulm Med. 2009; 15(1):57–62. [PubMed: 19077707]

98. Saito S, Iida A, Sekine A, et al. Identification of 779 genetic variations in eight genes encoding members of the ATP-binding cassette, subfamily C (ABCC/MRP/CFTR. J Hum Genet. 2002; 47(4):147–171. [PubMed: 12166651]

99. Weiss J, Theile D, Ketabi-Kiyanvash N, Lindenmaier H, Haefeli WE. Inhibition of MRP1/ABCC1, MRP2/ABCC2, and MRP3/ABCC3 by nucleoside, nucleotide, and non-nucleoside reverse transcriptase inhibitors. Drug Metab Dispos. 2007; 35(3):340–344. [PubMed: 17172311]

100. Mougey EB, Feng H, Castro M, Irvin CG, Lima JJ. Absorption of montelukast is transporter mediated: a common variant of OATP2B1 is associated with reduced plasma concentrations and poor response. Pharmacogenet Genomics. 2009; 19(2):129–138. [PubMed: 19151602]

101. Dahlin A, Litonjua A, Irvin CG, et al. Genome-wide association study of leukotriene modifier response in asthma. Pharmacogenomics J. 2015

102. Dahlin A, Litonjua A, Lima JJ, et al. Genome-Wide Association Study Identifies Novel Pharmacogenomic Loci For Therapeutic Response to Montelukast in Asthma. PLoS One. 2015; 10(6):e0129385. [PubMed: 26083242]

103. Shrestha Palikhe N, Kim SH, Jin HJ, Hwang EK, Nam YH, Park HS. Genetic mechanisms in aspirin-exacerbated respiratory disease. J Allergy (Cairo). 2012; 2012:794890. [PubMed: 21837245]

104. Lee EH, Park BL, Park SM, et al. Association analysis of RGS7BP gene polymorphisms with aspirin intolerance in asthmatic patients. Ann Allergy Asthma Immunol. 2011; 106(4):292–300. e296. [PubMed: 21457877]

105. Kim JH, Choi GS, Kim JE, et al. Clinical course of patients with aspirin-exacerbated respiratory disease: can we predict the prognosis? Pharmacogenomics. 2014; 15(4):449–457. [PubMed: 24624912]

106. Kohyama K, Abe S, Kodaira K, et al. Arg16Gly β2-adrenergic receptor gene polymorphism in Japanese patients with aspirin-exacerbated respiratory disease. Int Arch Allergy Immunol. 2011; 156(4):405–411. [PubMed: 21829036]

107. VandenBrink BM, Foti RS, Rock DA, Wienkers LC, Wahlstrom JL. Evaluation of CYP2C8 inhibition in vitro: utility of montelukast as a selective CYP2C8 probe substrate. Drug Metab Dispos. 2011; 39(9):1546–1554. [PubMed: 21697463]

108. Lu P, Schrag ML, Slaughter DE, Raab CE, Shou M, Rodrigues AD. Mechanism-based inhibition of human liver microsomal cytochrome P450 1A2 by zileuton, a 5-lipoxygenase inhibitor. Drug Metab Dispos. 2003; 31(11):1352–1360. [PubMed: 14570767]

109. Machinist JM, Mayer MD, Shet MS, Ferrero JL, Rodrigues AD. Identification of the human liver cytochrome P450 enzymes involved in the metabolism of zileuton (ABT-077) and its N-dehydroxylated metabolite, Abbott-66193. Drug Metab Dispos. 1995; 23(10):1163–1174. [PubMed: 8654206]

110. Karonen T, Laitila J, Niemi M, Neuvonen PJ, Backman JT. Fluconazole but not the CYP3A4 inhibitor, itraconazole, increases zafirlukast plasma concentrations. Eur J Clin Pharmacol. 2012; 68(5):681–688. [PubMed: 22108774]

111. Szczeklik A, Nizankowska E, Sanak M, Swierczynska M. Aspirin-induced rhinitis and asthma. Curr Opin Allergy Clin Immunol. 2001; 1(1):27–33. [PubMed: 11964666]

112. Shin SW, Park J, Kim YJ, et al. A highly sensitive and specific genetic marker to diagnose aspirin-exacerbated respiratory disease using a genome-wide association study. DNA Cell Biol. 2012; 31(11):1604–1609. [PubMed: 22994212]

113. Shin SW, Park JS, Park CS. Elevation of Eosinophil-Derived Neurotoxin in Plasma of the Subjects with Aspirin-Exacerbated Respiratory Disease: A Possible Peripheral Blood Protein Biomarker. PLoS One. 2013; 8(6):e66644. [PubMed: 23805255]

KEY POINTS

1. AERD severity and its clinical phenotypes are characterized by genetic variation within multiple pathways for arachidonic acid metabolism, inflammation and immune responses.
2. Epigenetic modifications, including DNA methylation and histone protein modification, contribute to regulation of many genes that contribute to inflammatory states in AERD.
3. The development of non-invasive, predictive clinical tests using data from genetic, epigenetic, pharmacogenetic and biomarker studies will improve precision medicine efforts for AERD and asthma treatment.

Immunol Allergy Clin North Am. Author manuscript; available in PMC 2017 December 07.

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*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
Airway cell function and response	*ADRB2*	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
	EMID2	EMID2_BL2_ht2	Differences in ΔFEV1 by aspirin provocation in AIA vs. ATA	163 AIA; 429 ATA; Asian (Korean)		CGAS	21086123
	KIF3A	rs3756775	Associated with the rate of FEV1 decline by aspirin provocation in AIA	103 AIA; 268 ATA; Asian (Korean)		CGAS	20922562
	SPINK5	G1258A, A1103G	Heterozygous genotypes were more frequently observed in AIA	15 CRSsNP; 59 CRSwNP (18 AIA); 30NC; Caucasian(European)		CGAS	22570283
Arachidonic acid metabolism and signaling	*LTC4S*	−444A>C	Association with ATA but not AIA	356 AIA; 840 ATA; 902 NC; Multiple (Caucasian, Asian, African-American)		MA	22884858
	ALOX5	ht1[GCGA]	Increased frequency of haplotype for AIA	93 AIA; 181 ATA; 123 NC; Asian (Korean)		CGAS	14749922
	COX2	−765G>C	CC homozygosity was associated with disease severity; increased prostaglandin production by monocytes	112 AIA; 198 ATA; 547 NC; Caucasian (Polish)		CGAS	15316498
	CYP2C19	rs4244285, rs4986893	Lower %predicted FEV1 following aspirin provocation in AERD	100 AERD; 300 ATA; 100 NC; Asian (Japanese)		CGAS	21855977

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s) or Functional Effect(s)	Study Population(s) and Ethnicities*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
	CYSLTR1	−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
	CYSLTR2	−819T>G, 2078C>T, 2534A>G	Increased frequency of minor alleles and decline in FEV1 by aspirin provocation in AIA	86 AIA; 134 ATA; 152 NC; Asian (Korean)		CGAS	15970796
	EP2	uS5, uS5b, uS7	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
	NAT2	−9246G>C	Associated with risk of AIA	170 AIA; 268 ATA; Asian (Korean)		CGAS	20602614
	PTGDR	−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; Caucasian (Spanish)		CGAS	23101307
	PTGER	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
	TBXA2R	rs11085026	Increased association and greater percent fall of FEV1 following aspirin provocation for AIA	93 AIA; 172 ATA; 118 NC; Asian (Korean)		CGAS	15898979
	TBXAS1	rs6962291	Lower frequency of minor allele; association with fall of FEV1 following aspirin provocation for AIA	200 AIA; 270 ATA; Asian (Korean)		CGAS	21449675
Inflammatory responses	*ACE*	−262A>T, −115T>C	Increased risk of AIA; homozygotes for minor alleles had a greater decline in FEV1 following aspirin provocation than reference homozygotes	81 AIA; 231 ATA; 181 NC; Asian (Korean)		CGAS	18727619

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s) or Functional Effect(s)	Study Population(s) and Ethnicities*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
	AGT	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
	CACNG6	rs192808; CACNG6_BL1_ht6	Associated with increased risk of AIA	102 AIA; 429 ATA; Asian (Korean)		CGAS	20860846
	CCR3	−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
	CRTH2	−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
	ECP		mRNA and protein expression increased in AERD	15 AERD; 15 CRSwNP; unspecified (United States)		EP	26067893
	ERAS		mRNA expression was increased in AIA vs. controls	11 AIA; 7 ATA; 15 NC; Caucasian (Polish)		EP	26646719
	FOSL1		mRNA and protein expression reduced in AIA vs. controls	11 AIA; 7 ATA; 15 NC; Caucasian (Polish)		EP	26646719
	HSPA1B	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
	IL13	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	162 AIA; 301 ATA; 430 NC; Asian (Japanese)			20358028

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s) or Functional Effect(s)	Study Population(s) and Ethnicities*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
			genotype of −1510A>C				
	NOS2A	[(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; Caucasian (Spanish)		CGAS	23101307
	PPARG	82466C>T	Increased MAF in AIA vs. ATA	60 AIA; 343 ATA; 449 NC; Asian (Korean)		CGAS	20224667
	RAB1A	14444 T>G, 41170 C>G	Increased MAF in AIA vs. ATA, and association with FEV1 decline following aspirin provocation	181 AIA; 1016 ATA; Asian (Korean)		CGAS	24555545
	SLC6A12	rs557881	Associated with AIA and decline in FEV1 following aspirin provocation	163 AIA; 429 ATA; Asian (Korean)		CGAS	20597903
	STK10	rs2306961	Associated with AIA	163 AIA; 429 ATA; Asian (Korean)		CGAS	21905501
Initiation of immune responses	*CIITA*	rs1139564	Associated with nasal polyposis in AERD	158 AERD; 309 ATA; Asian (Korean)		CGAS	23292525
	CNPY3		mRNA expression reduced in AIA vs. controls	11 AIA; 7 ATA; 15 NC; Caucasian (Polish)		EP	26646719
	GM-CSF		Elevated mRNA and protein in AERD	15 AERD; 15 CRSwNP; (United States)		EP	26067893
	HLADPB1	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	117 AERD; 685 ATA; Asian (Korean)	142 AERD; 946 ATA;	GWAS	23180272

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s) or Functional Effect(s)	Study Population(s) and Ethnicities*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
			percent decline in FEV1 following aspirin provocation		Asian (Korean)		
		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; Caucasian (Polish)		CGAS	9179433
	HLADPB2	exm-rs3129294	Predictive for AERD susceptibility vs. ATA	165 AERD; 397 ATA; 398 NC; Asian (Korean)		EWAS	25372592
	HLADQA1	DQA1*0301, DQA1*0201, DQA1*0501	Haplotypes associated with AERD risk	33 AERD; 17 ATA; 100 NC		CGAS	25975240
	HLADQB1	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
	HLADRB1	DRB1*04, DRB1*07, DRB1*011	Haplotypes associated with AERD risk	33 AERD; 17 ATA; 100 NC		CGAS	25975240
	HLADRB3		MAF lower for AERD vs. ATA	33 AERD; 17 ATA; 100 NC		CGAS	25975240
	HLADRB4		MAF higher for AERD vs. ATA	33 AERD; 17 ATA; 100 NC		CGAS	25975240
	IL10/TGFb	−1082A>G, −509C>T	−1082A/G associated with AIA; synergistic effect between TGF-β1−509C/T and IL-10−1082A/G in AIA	173 AIA; 260 ATA; 448 NC; Asian (Korean)		CGAS	19222424
	IL17RA	−1075A>G, −947A>G, −50C>T, BL1_ht1	MAF lower for AERD vs. ATA	143 AERD; 411 ATA; 825 NC; Asian (Korean)		CGAS	23220496
	IL4	−589T>C, −33C	Both associated with AIA risk; MAF for −589T>C was higher	103 AIA; 270 ATA; Asian (Korean)		CGAS	20921925

Pathway	Gene Symbol	Polymorphism(s)	Major Association(s), Clinical Phenotype(s) or Functional Effect(s)	Study Population(s) and Ethnicities*	Replication Population(s) and Ethnicities*	Study Type**	References (PMID)
			in the AIA vs. ATA and associated with a gene dose-dependent decline in FEV1 following aspirin provocation				
	IL5RA	−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
	MCP-1		Elevated mRNA and protein in AERD	15 AERD; 15 CRSwNP; unspecified (United States)		EP	26067893
	TAP2	multiple haplotypes	Associated with FEV1 decline by aspirin provocation in AERD	93 AERD; 96 ATA; Asian (Korean)		CGAS	21796142
	TLR3	−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
	UBE3C	rs3802122, rs6979947	Lower risk for AIA	163 AIA; 429 ATA; Asian (Korean)		CGAS	20934631
		rs10949635	Associated with nasal polyposis in ATA vs. AERD	114 AERD; 353 ATA; Asian (Korean)		CGAS	21881582
Asthma/NSAID response	*CEP68*	CEP68_ht4 (T-G-A-A-A-C-G), rs7572857	Associated with increased risk of AIA and higher decline of FEV1 following aspirin provocation	80 AIA; 100 ATA; Asian (Korean)	163 AIA; 429 ATA; Asian (Korean)	GWAS	21072201
	DPP10	rs17048175	Association with AERD but not ATA	274 AERD; 272 ATA; 99 NC; Asian (Korean)		CGAS	25592153

*Discovery/replication population and their race/ethnicity (if specified). AIA = aspirin intolerant asthma; AERD = aspirin exacerbated respiratory disease; ATA = aspirin tolerant asthma; NC = healthy normal controls; CRSwNP = chronic rhinosinusitis with nasal polyposis

** Type of study: CGAS = candidate gene association study; GWAS = genome-wide association study; MA = meta-analysis; EWAS = exome-wide association study; EP = expression profiling.