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. 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. 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. 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. Subsequent genetic studies revealed considerable evidence for genetic variation in AERD pathophysiology across multiple biological pathways, as well as variation in inter-individual treatment responses to multiple asthma drug classes including...
leukotriene modifiers and inhibitors\textsuperscript{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\textsubscript{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\textsuperscript{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\textsuperscript{2} and eosinophilia\textsuperscript{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\textsubscript{4}S 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 LTC\textsubscript{4}S\textsuperscript{16,17}, ALOX\textsubscript{5}\textsuperscript{18,19}, CYSLTR\textsubscript{1}\textsuperscript{20–22}, CYSLTR\textsubscript{2}\textsuperscript{20,21}, TBX2\textsuperscript{23}, EP\textsubscript{2}\textsuperscript{24}, and COX\textsubscript{2}\textsuperscript{25,26}. A summary of the clinical evidence for these associations are presented in Table 1. The arachidonic acid metabolism signaling pathway genes LTC\textsubscript{4}S, ALOX\textsubscript{5}, CYSLTR\textsubscript{1} and CYSLTR\textsubscript{2} represent the most important candidate genes in this pathway, and have the strongest evidence for a role in AERD pathogenesis. An LTC\textsubscript{4}S \textsuperscript{−444A/C} promoter SNP
(rs730012) is among the most widely reported variants associated with AERD, although its association with AERD across studies is inconsistent. 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). 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 (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. 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. 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. 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. 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. 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. 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. 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). Inflammatory mediators in the Th2 cytokine pathway may drive the development of symptoms characteristic of AERD, including chronic rhinosinusitis associated with nasal polyposis. 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 nasal polyps. However, the genetic and molecular mechanisms that
can differentiate this particular AERD phenotype from non-AERD phenotypes with nasal polypsis 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. AERD nasal polyps also contained significantly elevated protein levels of ECP, GM-CSF and MCP-1 as compared to the chronic rhinosinusitis samples, 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.

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. 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. However, this association did not persist after multiple test correction.

As eicosanoids and their receptors are upregulated in inflammatory cells within nasal polyp tissue, corresponding to high levels of LTC4, CYSLTR1 and PTGDR 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 polypsis 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). 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). 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. Modification of NOS2A transcript expression may be crucial for development of nasal polyps, 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; 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\textsuperscript{42,47}. Th2 cytokine IL-5 receptor alpha (\textit{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 \textit{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 \textit{IL5RA} SNPs (−5993G>A, −5567C>G and −5091G>A) and a case-control analysis and functional characterization of the SNPs were performed\textsuperscript{55}. AERD patients with \textit{IL5RA} −5993AA demonstrated a higher IgE to staphylococcal enterotoxin A ratio than heterozygotes or those possessing the reference allele\textsuperscript{55}. Furthermore, −5993A demonstrated altered promoter activity by luciferase reporter assay, and differential binding of nuclear extracts by EMSA\textsuperscript{55}. The authors conclude that \textit{IL5RA} −5993G>A may therefore contribute to eosinophil responses in AERD patients\textsuperscript{55}

In addition to IL5, evidence exists for the recently described, \textit{IL-17} cytokine family in inflammatory cell recruitment and allergic response\textsuperscript{56,57}. Polymorphisms in the \textit{IL-17A} receptor gene, \textit{IL17RA}, are associated with asthma, and \textit{IL-17A} activation induces activation of signaling molecules such as NF-KB that regulate inflammatory processes in human airway cells\textsuperscript{58}. In a recent candidate gene study in a Korean population, 15 SNPs in \textit{IL17RA} were analyzed and functionally characterized in 143 patients with AERD, 411 patients with ATA and 825 normal controls\textsuperscript{59}. Three \textit{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\textsubscript{1} following aspirin challenge, although the minor allele frequencies for all three SNPs were significantly lower for AERD\textsuperscript{59}. Finally, \textit{IL17RA} expression in CD14\textsuperscript{+} monocytes from asthmatic patients with all three minor allele genotypes for \textit{IL17RA} −1075A>G, −947A>G, −50C>T was significantly higher than for the reference homozygotes\textsuperscript{59}. The minor alleles of the three SNPs may therefore have protective effects for AERD, presumably by limiting \textit{IL17RA} expression\textsuperscript{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 \textit{HLA-DRB1*0301}, which is also associated with a higher leukotriene receptor antagonist dose to control symptoms and a higher prevalence of chronic rhinosinusitis\textsuperscript{60–62}. A recent genetic association study of \textit{HLA-DRB}, \textit{HLA-DQA1}, and \textit{HLA-DQB1} genotypes in 33 patients with AERD, 17 patients with ATA and 100 healthy controls was performed following an oral aspirin challenge\textsuperscript{63}. In comparison to the controls, frequencies of \textit{HLA-DQB1*0302} and \textit{HLA-DRB1*04} and the haplotypes \textit{HLA-DRB1*04/DQA1*0301/DQB1*0302} and \textit{HLA-DRB1*07/DQA1*0201/DQB1*0201} were higher in patients with AERD while \textit{HLA-DQB1*0301}, \textit{HLA-DQA1*0501}, \textit{HLA-DRB1*11}, and \textit{HLA-DRB3} allele frequencies were significantly lower\textsuperscript{63}. Furthermore, in contrast to ATA patients, patients with AERD had lower frequencies of \textit{HLA-DQB1*0301} and \textit{HLA-DRB1*01}\textsuperscript{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\textsuperscript{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. Steinken 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-\(\gamma\) which was also determined as originating from eosinophils through flow cytometry and histological studies \(^64\). Furthermore, in addition to priming these eosinophils, IFN-\(\gamma\) also increased the expression of genes involved in leukotriene biosynthesis and CysLT secretion \(^64\). IFN-\(\gamma\) 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-\(\gamma\)\(^64,65\). These findings suggest that IFN-\(\gamma\) 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.
Finally, AERD patients demonstrated a significant variation in eosinophil count by HSP SNP genotype, whereas the aspirin tolerant group did not. 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, 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.

### 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. 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. 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. 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. 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. 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. None of the SNPs achieved genome-wide significance; however, rs2281389 near HLA-DPB1 was most strongly associated with AERD (OR = 2.41; p = 5.69×10^-6). The top 49 SNPs associated with AERD risk were also associated with significant decline of FEV1 following aspirin challenge. 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. 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. 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. 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.

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. After filtering and
quality control of genotype data, over 54,000 SNPs remained and were evaluated for
association with AERD risk. 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). 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. Three additional exonic
SNPs on HLA-DPB1 (exm537513, exm537522 and exm537523) were also present among
the top 20 SNPs. A prior GWAS 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. 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. 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). 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. 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. DNA methylation profiles were interrogated using the Illumina genome-wide methylation assay chip. 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. Pathway analysis of the hypomethylated genes indicated enrichment in proliferation and activation of immune cells, cytokine production, and immune and inflammatory responses. 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. In addition, two Th2 cytokine encoding genes, IL5RA and IL10, were also differentially methylated. 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. 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.

B. Functional epigenetics of AERD

The role of epigenetic targeting of PGE\textsubscript{2} pathway genes involved in the expansion of nasal polyps in AERD patients was recently investigated. 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\textsubscript{2} receptor by PGE\textsubscript{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\textsubscript{2} to PGE\textsubscript{2}, was hypermethylated in nasal polyp tissue from AERD subjects. In addition, fibroblasts from nasal polyps of patients with AERD have intrinsically lower expression of COX-2, PGE\textsubscript{2} and EP\textsubscript{2} receptor protein vs. aspirin-tolerant (AT) control subjects. Cahill et al. hypothesized that an intrinsic defect in EP\textsubscript{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. 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. In contrast to ATA, fibroblasts from AERD patients proliferated quickly and also demonstrated persistent growth in response to treatment with PGE\textsubscript{2}, as well as having reduced expression levels of the EP\textsubscript{2} receptor. In addition, in AERD samples, EP\textsubscript{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\textsubscript{2} promoter correlated strongly with baseline EP\textsubscript{2} mRNA expression levels. However, DNA methylation at the EP\textsubscript{2} promoter in fibroblasts was not significantly different, suggesting that histone modification was more likely to contribute to EP\textsubscript{2} expression in nasal fibroblasts in AERD. 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. In AERD, LT overproduction has serious consequences for symptom severity and progressive airway disease. AERD patients show significant reductions in lung function, as determined by measuring forced expiratory volume in 1 second (FEV1), compared to non-AERD asthmatics, and significantly higher baseline and post-aspirin 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. In addition, COX-1 inhibitors (including aspirin) remove a brake on 5-LO activation, thereby increasing the baseline overproduction of LTs in AERD patients. 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. The gold standard for diagnosis of AERD is oral aspirin challenge to provoke symptomatic response. During this response, cysLT production is dramatically increased, precipitating symptoms. After diagnosis, significant improvement in asthma symptoms and slowing of nasal polyp recurrence are achieved with aspirin desensitization and daily high-dose aspirin treatment. 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. 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. Candidate gene and genome-wide investigations of anti-LT responses implicate involvement of multiple genes, including ALOX5, ALOX5AP, LTC4S, CYSLTR1, CYSLTR2, ABCC1, and OATP2B1. Recently, we conducted the first pharmacogenomics GWAS studies of zileuton and montelukast 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 DPB1,7,4,103, 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. 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. 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, TGFβ 509C>T, TNFa 308G>A and HLADPB1*0301) was conducted. 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. 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. 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. 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.

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 FEV1 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. 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, zileuton is an inhibitor of CYP1A2 and substrate of CYP3A4, and zafirlukast is a substrate of CYP2C9. 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

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References


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.
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<td>Gene Symbol</td>
<td>Polymorphism(s)</td>
<td>Major Association(s), Clinical Phenotype(s) or Functional Effect(s)</td>
<td>Study Population(s) and Ethnicities*</td>
<td>Replication Population(s) and Ethnicities*</td>
<td>Study Type **</td>
<td>References (PMID)</td>
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<td></td>
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<td></td>
<td>percent decline in FEV1 following aspirin provocation</td>
<td>Asian (Korean)</td>
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<td>Increased risk of AERD; predictive for AERD susceptibility vs. ATA</td>
<td>165 AERD; 397 ATA; 398 NC; Asian (Korean)</td>
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<td>EWAS</td>
<td>25372592</td>
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<td>Increased MAF in AIA</td>
<td>59 AIA; 57 ATA; 48 NC; Caucasian (Polish)</td>
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<td>CGAS</td>
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<td>Predictive for AERD susceptibility vs. ATA</td>
<td>165 AERD; 397 ATA; 398 NC; Asian (Korean)</td>
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<td>EWAS</td>
<td>25372592</td>
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<tr>
<td>HLADPB2</td>
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<td>Haplotypes associated with AERD risk</td>
<td>33 AERD; 17 ATA; 100 NC</td>
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<td>CGAS</td>
<td>25975240</td>
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<tr>
<td>HLADQA1</td>
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<td>DQA1<em>0301, DQA1</em>0201, DQA1*0501</td>
<td>Lower MAF for AERD vs. ATA</td>
<td>33 AERD; 17 ATA; 100 NC</td>
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<td>HLADQB1</td>
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<td>DQB1*0301</td>
<td>Lower MAF in poor responders to aspirin desensitization</td>
<td>16 AERD</td>
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<td>HLADRB1</td>
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<td>DRB1<em>04, DRB1</em>07, DRB1*011</td>
<td>Haplotypes associated with AERD risk</td>
<td>33 AERD; 17 ATA; 100 NC</td>
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<td>HLADRB3</td>
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<td>MAF lower for AERD vs. ATA</td>
<td>33 AERD; 17 ATA; 100 NC</td>
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<td>HLADRB4</td>
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<td>MAF higher for AERD vs. ATA</td>
<td>33 AERD; 17 ATA; 100 NC</td>
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<td>CGAS</td>
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<td>IL10/TGFβ</td>
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<td>−1082A&gt;G, −509C&gt;T</td>
<td>−1082A/G associated with AIA; synergistic effect between TGF-β1−509C/T and IL-10−1082A/G in AIA</td>
<td>173 AIA; 260 ATA; 448 NC; Asian (Korean)</td>
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<td>CGAS</td>
<td>19222424</td>
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<td>IL17RA</td>
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<td>−1075A&gt;G, −947A&gt;G, −50C&gt;T, BL1_at1</td>
<td>MAF lower for AERD vs. ATA</td>
<td>143 AERD; 411 ATA; 825 NC; Asian (Korean)</td>
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<td>23220496</td>
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<td>IL4</td>
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<td>−589T&gt;C, −33C</td>
<td>Both associated with AIA risk; MAF for −589T&gt;C was higher</td>
<td>103 AIA; 270 ATA; Asian (Korean)</td>
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<td>CGAS</td>
<td>20921925</td>
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<td>Pathway</td>
<td>Gene Symbol</td>
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<td>in the AIA vs. ATA and associated with a gene dose-dependent decline in FEV1 following aspirin provocation</td>
<td>139 AERD; 171 ATA; 160 NC; Asian (Korean)</td>
<td>CGAS 23470716</td>
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<td>IL5RA</td>
<td>−5993G&gt;A</td>
<td>AA genotype had increased IgE responses to staphylococcal enterotoxin A in AERD patients</td>
<td>15 AERD; 15 CRSwNP; unspecified (United States)</td>
<td>EP 26067893</td>
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<td>MCP-1</td>
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<td>Elevated mRNA and protein in AERD</td>
<td>93 AERD; 96 ATA; Asian (Korean)</td>
<td>CGAS 21796142</td>
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<td>TAP2</td>
<td>multiple haplotypes</td>
<td>Associated with FEV1 decline by aspirin provocation in AERD</td>
<td>203 AERD; 254 ATA; 274 NC; Asian (Korean)</td>
<td>CGAS 21461252</td>
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<td>TLR3</td>
<td>−299698G&gt;T, 293391G&gt;A</td>
<td>Increased MAF in AERD vs. ATA for −299698G&gt;A'; reduced MAF 2200698G&gt;T in AERD vs. ATA</td>
<td>2200698G&gt;T in AERD vs. ATA</td>
<td>CGAS 21072201</td>
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<td>UBE3C</td>
<td>rs3802122, rs6979947</td>
<td>Lower risk for AIA</td>
<td>163 AIA; 429 ATA; Asian (Korean)</td>
<td>CGAS 20934631</td>
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<td>rs10949635</td>
<td>Associated with nasal polyposis in ATA vs. AERD</td>
<td>114 AERD; 353 ATA; Asian (Korean)</td>
<td>CGAS 21881582</td>
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<td>Asthma/NSAID response</td>
<td>CEP68</td>
<td>CEP68_ht4 (T-G-A-A-A-C-G), rs7572857</td>
<td>Associated with increased risk of AIA and higher decline of FEV1 following aspirin provocation</td>
<td>80 AIA; 100 ATA; Asian (Korean)</td>
<td>GWAS 21072201</td>
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<td>DPP10</td>
<td>rs17048175</td>
<td>Association with AERD but not ATA</td>
<td>274 AERD; 272 ATA; 99 NC; Asian (Korean)</td>
<td>CGAS 25592153</td>
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</table>

* 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.