Brief report

Aspirin-exacerbated respiratory disease is associated with variants in filaggrin, epithelial integrity, and cellular interactions

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Background: Previous studies have determined that up to 6% of patients with aspirin-exacerbated respiratory disease (AERD) have family history of AERD, indicating a possible link with genetic polymorphisms. However, whole exome sequencing (WES) studies of such associations are currently lacking.
Objectives: We sought to examine whether WES can identify pathogenic variants associated with AERD.
Methods: Diagnoses of AERD were confirmed in patients with nasal polyps and asthma. WES was performed using an Illumina sequencing platform. Human Phenotype Ontology terms were used to define the patients' phenotypes. Exomiser was used to annotate, filter, and prioritize possible disease-causing genetic variants.
Results: Of 39 patients with AERD, 41% reported a family history of asthma and 5% reported a family history of AERD. Pathogenic exome variants in the filaggrin gene (*FLG*) were found in 2 patients (5%). Other variants not known to be pathogenic were detected in an additional 16 patients (41%) in genes related to epithelial integrity and cellular interactions, including genes encoding desmoglein 3 (*DSG3*), dynein axonemal heavy chain 9 (*DNAH9*), collagen type VII alpha 1 chain (*COL7A1*), collagen type XVII alpha 1 chain (*COL17A1*), chromodomain helicase DNA binding protein-7 (*CHD7*), TSC complex subunit 2/tuberous sclerosis-2 protein (*TSC2*), P-selectin (*SELP*), and platelet-derived growth factor receptor-alpha (*PDGFRA*).
Conclusion: WES identified a monogenic susceptibility to AERD in 5% of patients with *FLG* pathogenic variants. Other variants not previously identified as pathogenic were found in genes relevant to epithelial integrity and cellular interactions and may further reveal genetic factors that contribute to this condition.
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Key words: *Aspirin-exacerbated respiratory disease, NSAID-ERD, AERD, filaggrin, epithelial barrier, aspirin*

Abbreviations used
AERD: Aspirin-exacerbated respiratory disease
N-ERD: Nonsteroidal anti-inflammatory drug–exacerbated respiratory disease
NSAID: Nonsteroidal anti-inflammatory drug
WES: Whole exome sequencing

INTRODUCTION

Aspirin-exacerbated disease (AERD), also known as nonsteroidal anti-inflammatory drug (NSAID)-exacerbated respiratory disease (N-ERD), is a subset of eosinophilic asthma associated with recurrent nasal polyposis and acute respiratory reactions to COX-1 inhibitors such as aspirin and other NSAIDs.[1] Nasal polyps and aberrant arachidonic acid metabolism are the core contributors to this syndrome.[1-3] There is little familial accumulation of AERD, with no more than 6% of patients reporting a family history of the syndrome.[4,5] However, at least 1 study reported a family history of asthma in almost a half of patients with AERD.[6] To test associations between AERD and genetic variability, multiple genome-wide association studies have revealed possible links between AERD status and polymorphism in genes responsible for cysteinyl-leukotriene signaling.[7]

Whole exome sequencing (WES) is used for the detection of rare genetic variants in all coding sequences of an individual. It is estimated that 85% of the disease-causing mutations are defined in protein-coding regions of the genome. For this reason, sequencing of all exons (the "exome") can uncover the causes of monogenic disorders as well as predisposing variants for common diseases.[8] For most common and complex diseases, polygenic inheritance involving several common genetic variants of small effect plays a more recognizable role than rare monogenic mutations do.[9] Exome sequencing offers the opportunity to detect rare protein-coding variants causing monogenic susceptibility to a disease. The analysis of the individual variants revealed by WES combines findings of damage to the gene with a match to the expected phenotype.[10] This kind of analysis is performed using Exomiser, a software application that prioritizes genes and variants in sequencing studies.[11] The Exomiser approach appears to increase the diagnostic success of WES.[12,13]

Using Exomiser and a phenotype-driven approach, we sought to examine whether WES can identify variants associated with

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TABLE I. Patient characteristics

Characteristic	All patients (N = 39)	Patients with family history of asthma (n = 16)	Patients with no family history of asthma (n = 23)	*P* value
Age (y), median (IQR)	45 (34-57)	40 (34-51)	48 (34-57)	.4
Sex (women/men), no. (%)	24/15 (62/38)	11/5 (69/31)	13/10 (57/43)	.5
Race, no. (%)				.09
Black	19 (49)	10 (63)	9 (39)	
Latino	9 (23)	5 (31)	4 (17)	
White	9 (23)	1 (6)	8 (35)	
Asian	2 (5)		2 (9)	
Use of ICS/LABA, no. (%)	36 (76)	15 (93)	21 (91)	.9
Long-term oral prednisone use, no. (%)	5 (13%)	5 (31)	0	**<.01**
Baseline FEV1 value (%), predicted ± SE	80 (± 2.6)	79 (± 4.0)	82 (± 3.6)	.6
Baseline peripheral blood eosinophil concentration (K/μL), median (IQR)	0.9 (0.7-1.2)	1.0 (0.8-1.4)	0.8 (0.6-1.1)	.3
Baseline total serum IgE level (IU/mL), median (IQR); mean (± SE)	351 (84-802); 519 (± 103)	802 (171-1035); 847 (± 197)	210 (84-366); 256 (± 47)	**<.01**
Lund-Mackay chronic rhinosinusitis severity score, median (IQR)	20 (16-22)	21 (18-22)	20 (16-22)	.6
Age of asthma onset (y), median (IQR)	35 (26-44)	30 (23-39)	40 (32-46)	.09
Age of polyp onset (y), median (IQR)	39 (30-46)	37 (27-45)	44 (30-47)	.5
Age of onset of NSAID allergy (y), median (IQR)	39 (30-48)	39 (27-43)	45 (30-51)	.2
Family history of N-ERD, no. (%)	2 (5)	1 (6)	1 (4)	.8
Presence of pathogenic exome variants on WES, detected by Exomiser (yes/no), no. (%)	2/39 (5)	1/16 (6)	1/23 (4)	.8
Pollution exposure (yes/no), no. (%)*	18/10 (64%)	12/2 (86%)	6/8 (43%)	**.02**
Secondhand smoking (yes/no), no. (%)†	8/26 (38%)	7/9 (44%)	1/17 (6%)	**.01**
Any smoking (yes/no), no. (%)‡	13/21 (38%)	9/7 (56%)	4/14 (22%)	**.04**

Boldface indicates statistical significance.

ICS, Inhaled corticosteroid; *IQR*, interquartile range; *LABA*, long-acting β-agonist.

*Patient-reported pollution exposure defined as a history of excessive exposure to car exhaust, occupational exposure to chemicals at work on railroads, automobile mechanic shops, paint, construction dust, and/or home's proximity to highways and bus depots.

†Patient-reported exposure to second-hand smoking before development of N-ERD.

‡Patient-reported history of smoking prior to development of N-ERD (none of the patients in the cohort were current smokers).

AERD and a family history of AERD in a demographically diverse cohort of patients from the Bronx borough of New York City.

RESULTS AND DISCUSSION

A previously reported protocol involving aspirin challenge was used to diagnose AERD in the study participants with asthma and nasal polyps.[14] Family history of AERD and asthma was self-reported by the study participants. Genomic DNA was purified from nasal polyp tissues for WES. Library preparation with exome enrichment, Illumina sequencing, and variant calling was performed by Novogene, Inc (Sacramento, Calif). We restricted our search for disease-causing variants to genetic alterations in coding regions that were both rare and likely pathogenic. Pathogenicity of gene variants was classified through the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/). We used Exomiser, a tool that utilizes both patient-specific clinical/phenotypic data (encoded in the form of Human Phenotype Ontology [HPO] terms) and patient-specific genetic variants to annotate, filter, and prioritize likely disease-causing exome variants and their associated genes.[10] To comprehensively characterize this cohort, the following HPO terms were used for the search: *aspirin-induced asthma* (HP:0012042), *nasal polyposis* (HP:0100582), *eosinophilia* (HP:0001880), *hypereosinophilia* (HP:0032061) and *increased circulating IgE levels* (HP:0003212). The variant and phenotype scores were combined to generate a single, gene-specific Exomiser score. A higher Exomiser score indicates a greater likelihood of a pathogenic variant that is consistent with the patient's clinical features. Genes with Exomiser scores higher than 0.8 that appeared in at least 2 patients were considered as initial candidates (for additional details, see the Supplementary Methods in the Online Repository at www.jaci-global.org).

A total of 39 patients with AERD were enrolled in the study. Their demographic and clinical characteristics were compared from the standpoint of presence of a family history of asthma (Table I). Most of the patients (62%) were women. Nearly half self-identified as Black (49%), and 23% identified as Latinos from the Dominican Republic or Puerto Rico. Two patients (5%) reported a family history of AERD. Patients with a family history of asthma (41%) had a higher baseline IgE level, were more likely to take oral corticosteroids, and were more likely to report excessive environmental pollution exposure as well as any smoking.

The presentations of 28 patients (72%) fit all 5 HPO terms: aspirin-induced asthma, nasal polyposis, eosinophilia, hypereosinophilia, and increased circulating IgE levels. In all, 8 patients (21%) fit 4 HPO terms, 1 patient (5%) fit 1 HPO term, and 1 patient (2%) fit 1 HPO terms (Table II).

Exome variants

Pathogenic exome variants were found in 2 of 39 patients (5% [Table III]). In both patients, these variants were found in the filaggrin (*FLG*) gene. The identified variants were heterozygous. There was no significant association between family history of asthma and the presence of these gene variants (Table I). Both

TABLE II. Characteristics of the groups identified through patient phenotype

Cohort characteristic (N = 39)	Group of 28 (aspirin-induced asthma, nasal polyposis, eosinophilia, hypereosinophilia, elevated serum IgE levels)	Group of 8 (aspirin-induced asthma, nasal polyposis, eosinophilia, hypereosinophilia	Group of 2 (aspirin-induced asthma, nasal polyposis)	Group of 1 (aspirin-induced asthma, nasal polyposis, elevated serum IgE levels)
Age (y), median (± SE)	43 (± 2.5)	53 (± 5.8)	48 (± 6.5)	31
Sex (women/men), no. (%)	16/13 (55/45)	6/2 (75/25)	2/0 (100)	Male
Baseline peripheral blood eosinophil concentration (K/μL), median (IQR)	1.0 (0.8-1.25)	0.8 (0.7-0.9)	0.15 (0.1-0.2)	0.2
Baseline total serum IgE level (IU/mL), median (IQR); mean (± SE)	462 (255-896) 668 (± 119)	62 (44-76) 58 (± 8.5)	45 (20-70) 45 (± 25)	367
Pathogenic variants with an Exomiser score of 0.8 or higher				
FLG aggregates keratin intermediate filaments	2 of 28 patients (7%)			

IQR, Interquartile range.

variants were identified as pathogenic variants and resulted in the loss of protein function, according to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/).

Other variants included nonpathogenic variants in *FLG* and in the following 8 genes: TSC complex subunit 2/tuberous sclerosis 2 protein (*TSC2*), selectin P (*SELP*), platelet-derived growth factor receptor alpha (*PDGFRA*), desmoglein 3 (*DSG3*), dynein axonemal heavy chain 9 (*DNAH9*), collagen type VII alpha 1 chain (*COL7A1*), collagen type XVII alpha 1 chain (*COL17A1*), and chromo-domain helicase DNA binding protein 7 (*CHD7*) (see Table E1 in the Online Repository at www.jaci-global.org). The variants in these genes were detected in 7% to 11% of patients who met all or most of the examined HPO terms. No gene variants were detected in 3 patients who met only 2 or 3 HPO terms (see Table E2 in the Online Repository at www.jaci-global.org). The variants detected in each patient are summarized in Table E3 (available in the Online Repository at www.jaci-global.org). The role of the nonpathogenic variants in N-ERD requires further investigation. Their pathogenicity scores are shown in Table E1.

In this study, we assessed coding sequence variant associations with AERD status in a well-phenotyped group of patients. We used the latest version of the bioinformatics tool Exomiser to perform phenotype-driven variant prioritization analysis of whole exome data using clinical relevance and a wide range of other computational filters for variant frequency and predicted pathogenicity.[10]

More than one-third (41%) of the study participants reported a family history of asthma. In 2 patients (5%), we detected 2 pathogenic exome variants in *FLG*. Both pathogenic variants were identified in patients with a reported family history of N-ERD (Table III).

Inheritance in AERD is rare but not unknown.[4,5] It was not possible to test for AERD in family members of the 2 patients with pathogenic mutations in our cohort. This approach could be considered in future studies. *FLG* plays a key role in epithelial barrier function and is associated with type 2 inflammatory disorders, such as asthma and atopic dermatitis.[15,16] Although both of the aforesaid patients had asthma in the setting of AERD, they had no evidence of atopic dermatitis, even in childhood, although this was self-reported. At least 1 study reported *FLG* mutations in 25% of patients with nasal polyps, although their AERD diagnosis is unknown.[17]

Other Exomiser-detected variants in this study were not classified as pathogenic on ClinVar. Notably, these variants were identified in genes whose function is associated with epidermis development and cell interactions. In addition to *FLG* variants, the other prioritized exome variants encode proteins involved in tissue integrity, such as *COL17A1* (possible role in desmosome integrity), *COL7A1* (epithelial basement membrane protein), *DSG3* (desmosome junction component), and *DNAH9* (ciliary function in respiratory epithelia). Multiple dynein genes (*DNAH5, DNAH8, DNAH9,* and *DNAH11*) have been associated with chronic rhinosinusitis.[18] The role of dynein genes in nasal polyposis or AERD has not been established.

One additional group of detected gene variants in our study may be linked to a previously reported role of cellular associations in AERD.[19] *PDGFRA* encodes a tyrosine protein kinase that plays a role in cell migration and chemotaxis, whereas *SELP* encodes P-selectin, which mediates rolling of leukocytes over vascular surfaces and the interaction of endothelial cells or platelets with leukocytes. These platelet-leukocyte interactions may play a role in the predominance of type 2 inflammation that is characteristic for AERD.[19]

Previously reported gene polymorphisms in AERD suggested a variety of genetic associations contributing to the disease's onset when environmental conditions are met.[7,18] AERD was associated with a downregulation of genes responsible for the epithelial barrier function.[20] To date, the only exonic variant study in a Korean population using the Exome BeadChip assay indicated that a combination of exonic SNPs within the HLA genes was associated with AERD.[21] A probable explanation for the array of candidate genes associated with AERD could be the complexity of the interpretation of the wealth of variants found, as well as the incomplete knowledge of gene function.[22,23]

To our knowledge, this is the first study using WES to identify protein-coding variants associated with a monogenic susceptibility for AERD in 5% of the patients. A strength of our study is the diverse and well-characterized population of patients with AERD, which includes a high percentage of Black and Latino individuals. Another strength is the novel use of Exomiser for a phenotype-driven prioritization of genetic variants, which may become a valuable tool for clinical evaluations of genetic associations in the future.

Limitations of the study include a modest sample size, which may account for the lack of associations between reported gene variants and clinical or demographic features. In addition, self-reporting of history may be fraught with recall bias.

TABLE III. Pathogenic exome variant details: Genes with an Exomiser score of 0.8 or higher, pathogenic variants

Gene	rs ID of SNP	HGVS nomenclature	ClinVar	ACMG class	Varsome	Patient description	Gene function
FLG	rs200002200	chr1:152308777 G->A; Arg2037* Stop gained	https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000280166.10	Pathogenic	https://varsome.com/variant/hg38/rs200002200?annotation-mode=germline	28-year-old Latino man; *FLG* variant was the only variant identified in this patient	Aggregates keratin intermediate filaments and promotes disulfide bond formation among the intermediate filaments during terminal differentiation of mammalian epidermis https://www.genecards.org/cgibin/ carddisp.pl?gene=FLG&keywords= flg#function
	rs61816761	chr1:152313385 G->A; Arg501* Stop gained	https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000016319.50	Pathogenic	https://varsome.com/variant/hg38/rs61816761?annotation-mode=germline	56-year-old White woman; 2 variants have been identified: an *FLG* variant and a benign variant in the *TSC2* gene	

ACMG, American College of Medical Genetics and Genomics; *HGVS,* Human Genome Variation Society; *ID,* identifier; *SNP,* single-nucleotide polymorphism.

In summary, this study identified 2 pathogenic variants in *FLG* associated with AERD. To date, additional identified variants are classified as nonpathogenic; however, their role in AERD requires further investigations. Our observations that genetic variants responsible for epithelial integrity and for cellular interactions indicate plausible pathways associated with AERD.

DISCLOSURE STATEMENT

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Key messages

- **Whole exome sequencing detected pathogenic variants in the *FLG* gene in a subset of patients with AERD. Other sequence variants were found in a variety of genes relevant to epithelial integrity and cellular interactions.**
- **Family history of asthma was reported by 41% of patients with AERD. Family history of AERD was reported by 5% of these patients, which is consistent with previous reports.**

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