REVIEWS

Asthma Exacerbations: The Genes Behind the Scenes

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Abstract
The clinical and socioeconomic burden of asthma exacerbations (AEs) constitutes a major public health problem. In the last 4 years, there has been an increase in ethnic diversity in candidate-gene and genome-wide association studies of AEs, which in the latter case led to the identification of novel genes and underlying pathobiological processes. Pharmacogenomics, admixture mapping analyses, and the combination of multiple “omics” layers have helped to prioritize genomic regions of interest and/or facilitated our understanding of the functional consequences of genetic variation. Nevertheless, the field still lags behind the genomics of asthma, where a vast compendium of genetic approaches has been used (eg, gene–environment interactions, next-generation sequencing, and polygenic risk scores). Furthermore, the roles of the DNA methylome and histone modifications in AEs have received little attention, and microRNA findings remain to be validated in independent studies. Likewise, the most recent transcriptomic studies highlight the importance of the host–airway microbiome interaction in the modulation of risk of AEs. Leveraging -omics and deep-phenotyping data from subtypes or homogenous subgroups of patients will be crucial if we are to overcome the inherent heterogeneity of AEs, boost the identification of potential therapeutic targets, and implement precision medicine approaches to AEs in clinical practice.

Key words: Asthma exacerbations. Genomics. Epigenetics. Transcriptomics.

Resumen
La carga clínica y socioeconómica de las exacerbaciones asmáticas (EA) representa un importante problema de salud pública. En los últimos cuatro años, ha aumentado la diversidad étnica en los estudios de asociación de genes candidatos y del genoma completo (GWAS) de las EA, lo que, en este último caso, ha llevado a la identificación de nuevos genes y procesos fisiopatológicos subyacentes. La farmacogenómica, el análisis de mapeo por mezcla y la combinación de múltiples capas “ómicas” han contribuido a priorizar regiones genómicas de interés y/o comprender las consecuencias funcionales de la variación genética. A pesar de esto, el campo todavía está en desarrollo en comparación con la genómica del asma, donde se ha utilizado un amplio compendio de enfoques genéticos (por ejemplo: interacciones gen-ambiente, secuenciación de nueva generación o puntuaciones de riesgo poligénico). Además, el papel de la metilación del ADN y las modificaciones de las histonas en las EA se ha explorado escasamente, y los hallazgos relacionados con los microARNs aún no se han validado en estudios independientes. Asimismo, los estudios transcriptómicos más recientes destacan la importancia de la interacción entre el microbioma de las vías respiratorias y el huésped en la modulación del riesgo de las EA. La integración de datos ómicos y de fenotipado profundo de subtipos o subgrupos homogéneos de pacientes será crucial para superar la heterogeneidad inherente de las EA e impulsar la identificación de dianas terapéuticas potenciales y la implementación de la medicina de precisión para las EA en la práctica clínica.

Introduction

Asthma exacerbations (AEs) are episodes of worsening symptoms requiring a change in treatment. AEs can be severe, and while multiple criteria have been used in the literature [1], a common definition comprises asthma-related hospitalizations, emergency department (ED) visits, and the use of systemic corticosteroids (oral, intramuscular, or intravenous). However, this definition should be used with caution because it often relies on an individual’s self-report and does not consider other clinical or physiological parameters underlying the episode [2].

AEs are a major public health problem and a priority in asthma research. Each year, approximately 75 000 people are hospitalized and 15 000 people die from asthma in the United Kingdom [3]. In the United States of America (USA), there are approximately 170 000 asthma-related hospitalizations, 1.8 million ED visits, and 4000 asthma-related deaths per annum. In fact, yearly asthma health care expenditure amounts to £1.1 billion in the United Kingdom [4] and $50.3 billion in the USA [5]. Indirect asthma costs, such as work and school absences, further increase the economic impact of asthma [5].

Moreover, AEs affect the quality of life of individuals with asthma [6,7] and their caregivers [8]. Although several studies have found an association between AEs and decline in lung function [9-13], infants with reduced airway caliber may also be at higher risk of loss of lung function and AEs [14,15]. In fact, the baseline airway wall area percent, an indicator of airway remodeling, is associated with the annual rate of future AEs and long-term decline in lung function [16,17].

To date, the best predictor of AEs is having had one within the previous year [18-20], thus, highlighting the need to investigate the key roles of genetic factors and/or early-life determinants on these events. In addition, identifying clinically relevant biomarkers or predictors of AEs plays a key role in reducing and preventing them. AEs are likely due to the complex interplay of genetic, environmental, and behavioral factors [18,21-23]. In fact, risk factors for AEs include allergens, air pollution, exposure to tobacco smoke, viral infections, psychological stress, poor adherence to treatment, obesity, and genetic factors [18].

Ethnic differences in the patterns of AEs are evidenced worldwide. In the USA, AE rates are higher among African Americans and Puerto Ricans [24-28], while in Europe, they are higher in southern European countries [29]. African Americans are also more likely to have longer lengths of stay in intensive care units than Europeans [30]. In fact, African ancestry has been associated with AEs among African Americans [31] and other individuals of African descent in the USA [32]. However, this association has not been validated for the number of exacerbations [33] or in Puerto

Definitions

- Candidate-gene association study: Statistical approach that interrogates the association between genetic variation and a trait of interest and analyzes genomic regions selected based on a biological hypothesis.
- Genome-wide association study (GWAS): Agnostic scan of genetic variation across the genome to establish an association with a trait of interest.
- Genome-wide interaction study (GWIS): Agnostic scan of the interaction between genetic variation across the genome and a factor of interest (eg, environmental or genetic factors) in a phenotype under study.
- Admixture mapping: Gene mapping approach that investigates whether chromosomal ancestry (local ancestry) is associated with a trait of interest, thus enabling the detection of genomic regions harboring genetic variants that exhibit differences in ancestry.
- Next-generation sequencing (NGS): High-throughput technology that makes it possible to determine the DNA sequence of single DNA molecules in parallel. These methods involve DNA fragmentation, DNA sequencing, and mapping to an organism’s reference genome to detect the genetic variation of a given sample.
- Whole-exome sequencing (WES): Next-generation approach that makes it possible to detect genetic variation in the genomic protein-coding regions (exons).
- Whole-genome sequencing (WGS): Next-generation approach that determines the DNA sequence in the entire genome.
- Epigenome-wide association studies (EWAS): Agnostic scan of epigenetic markers, usually DNA methylation, across the genome to determine association with a trait of interest.
- Transcriptome-wide association studies (TWAS): Agnostic gene-based scan of gene expression across the whole genome to determine association with a trait of interest.
- Proteome-wide association studies (PWAS): Agnostic scan of protein expression to determine association with a trait of interest.
- Metabolome-wide association studies (MWAS): Agnostic scan of metabolite levels to determine association with a trait of interest.
- Quantitative trait locus (QTL): Position of the DNA sequence where genetic variation is associated with a quantitative trait, such as DNA methylation (methylation quantitative trait locus, meQTL), gene expression levels (expression quantitative trait locus, eQTL), splicing ratios of transcripts (splicing quantitative trait locus, sQTL), protein levels (protein quantitative trait locus, pQTL), and metabolite levels (metabolic quantitative trait locus, mQTL).
- Expression quantitative trait methylation (eQTM): Position of the DNA sequence where methylation levels are associated with gene expression levels.
Ricans [34], a recently admixed population with up to 25% African ancestry [34,35]. More recent findings suggest that the association between African ancestry and readmissions for asthma in African Americans may be mediated by disease management and socioeconomic factors [36].

Herrera-Luis et al [16] provided a detailed description of genetic association studies of AE published until November 15, 2018. Gautam et al [37] reviewed the transcriptomics of asthma susceptibility, disease severity, and AEs prior to 2022, although no extensive summary of epigenetic studies of AEs has been published to date. In this review, we discuss the latest findings from -omics studies of AEs, assess methodological challenges, and propose future directions in this research field. For that purpose, we provide an update on the state of the art of the genomics and transcriptomics of AEs from the aforementioned dates to October 1, 2022 and review epigenetic aspects of AEs.

**Literature Mining**

OR Genetics [Title/Abstract] OR GWAS [Title/Abstract] OR GWAS [Title/Abstract] or epigenetic [Title/Abstract] OR methylation [Title/Abstract] OR histone [Title/Abstract] OR micro RNA [Title/Abstract] OR mRNA [Title/Abstract] OR transcriptomics [Title/Abstract] AND (asthma with exacerbations [Title/Abstract]). We excluded manuscripts reporting the following: (1) reviews, editorials, or opinion articles; (2) findings in animals or cell lines; and (3) no data on -omics or candidate-gene associations with AEs.

Genetic Association Studies

The genetic determinants of AEs have been thoroughly investigated using hypothesis-driven approaches to select genomic regions of interest [1] (Figure, A). However, candidate-gene association studies are hampered by nonreproducibility across studies and a low likelihood of identifying true biological risk variants because of the polygenic structure underlying complex human traits [39]. Conversely, genome-wide association studies (GWAS) allow for agnostic assessment of genetic variation across the genome for association with a trait. These hypothesis-free strategies can uncover novel pathogenic mechanisms, potentially leading to new therapeutic targets [40] (Figure, B). Most genetic association studies have investigated single-nucleotide polymorphisms (SNPs), which are base substitutions at a single position in the genome sequence. Although rare genetic variations (<1% minor allele frequency) may be implicated in the pathophysiology of AEs, whole-genome or exome association studies have yet to be conducted for AEs (Figure, C).

In populations resulting from the admixture of 2 or more ancestral populations, admixture mapping analysis can be an alternative strategy that avoids the high penalty of statistical significance in GWASs, particularly in genetically complex populations, which are often underrepresented in biomedical research [41-43]. Briefly, differences in the number of copies of alleles inherited from distinct ancestral populations at a given locus, or “local ancestry”, can be leveraged to distinguish candidate regions where local ancestry is associated with a trait of interest (Figure, D). Genetic variants within the biologically plausible candidate region are then interrogated for association with the trait to identify causal variants that usually evince distinct allele frequencies between ancestral populations [41,42,44].

Since the most frequently used approaches to identify susceptibility alleles for AEs are biased toward uncovering variants with modest effect sizes (ie, candidate gene studies) or located in noncoding regions of the genome (ie, GWAS), it is imperative to comprehensively assess the functional impact of genetic variation [1,45]. In this context, investigating the effect of variants on different -omics layers (Figure, E) has become easier, given the availability of multiple free tools online (see [46-49]).

Candidate-gene association studies

Most candidate-gene association studies of AEs focused on polymorphisms in genes previously implicated in asthma or in viral pathways [1], such as interleukin 33 (IL33) [50], vitamin D receptor (VDR) [54], and SERPINE1 encoding the plasminogen activator inhibitor-1 (PAI-1) [52] (Table 1). For instance, adding several asthma-related variants at SPATS2L and IL33 that were associated with ED management failure to ED-related clinical scores improved the ability to predict ED management failure compared with the clinical model alone (area under the curve [AUC]: 0.82 vs 0.79, \( P=0.004 \)) [50]. Moreover, an expression quantitative trait loci analysis of respiratory syncytial virus–related genes narrowed down the modulatory effect of respiratory syncytial virus infection on a CEACAM3 locus for AEs [53]. Likewise, a candidate-gene association study of 6 genomic regions harboring genes whose combined sputum gene expression signature exhibited predictive capability for exacerbations uncovered a DNASE1L3 locus for AEs associated with DNASE1L3 transcript expression levels in asthma-related tissues [56].

The first GWAS of asthma revealed variants at chromosome 17q12-21, with larger effects on asthma in children than in adults [64] and whose effects may be intensified by early-life and passive tobacco smoke exposure [65,66]. As expected for the most consistently replicated signal of asthma across populations, genetic variation and gene-by-environment interactions for chromosome 17q12-21 have been investigated in relation to AEs [1,67]. More recently, the effect of the GSDMB rs7216389 SNP on AEs was found not to be modulated by prenatal second-hand smoke exposure in Danish children [68]. Several 17q12-21 variants are associated with expression levels of nearby genes in bronchial epithelial cells and located within binding sites for interferon regulatory factors, suggesting effects through antiviral pathways [59], consistent with previous gene-by-environment evidence on asthma susceptibility [67].

Two recent studies of Korean individuals with asthma revealed genetic associations for AEs in NLRP4 and OXSR1 that differed by smoking status [51,62]. NLRP4 is a regulator of the inflammasome that acts as an inhibitor of type I interferon signaling, tumor necrosis factor (TNF) α, and IL-1β–mediated NF-κB activation [69]. Conversely, OXSR1 encodes an oxidative stress responsive kinase that participates in ion transport and cell volume homeostasis [62]. In fact, OXSR1 expression was increased by smoke exposure and corticosteroid treatment in various airway cell types [62].

In the period reviewed, 4 candidate gene studies explored susceptibility variants for response to inhaled corticosteroids (ICS) [60,61,63,70], long-acting β2-agonists [55], and montelukast [58] using AEs as a clinical endpoint. Four of them assessed asthma-related genes, as follows: IL1RL1 [60] and CRHR1 [61] for response to ICS, ADRB2 [55] for response to long-acting β2 agonists, and LTA4H [58] for response to montelukast. The other 2 combined or integrated multiple -omics to prioritize candidate genes [63,70]. Hernandez-Pacheco et al [57] identified a member of the family of latent-transforming growth factor β–binding proteins (LTPB1) as differentially expressed after exposure to corticosteroids in several transcriptomic datasets from away smooth muscle cells and peripheral blood mononuclear cells. Within LTPB1, 2 polymorphisms associated with AEs exerted ethnicity-specific effects [70]. Kan et al [63] leveraged a previous GWAS of change in forced expiratory volume in 1 second (FEV₁) after ICS treatment (\( P<1\times10^{-6} \)), chromatin immunoprecipitation
Table 1. Main Findings and Characteristics of Candidate-Gene Studies of Asthma Exacerbations Conducted From November 15, 2018 to October 1, 2022

<table>
<thead>
<tr>
<th>rsID (Gene)</th>
<th>Participants</th>
<th>Phenotype</th>
<th>EA/EG</th>
<th>Effect size (95%CI) or (SE)</th>
<th>P Value</th>
<th>PMID [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs295137 (SPATS2L)</td>
<td>491 European children with moderate-to-severe asthma presenting to the ED</td>
<td>Hosp/active asthma management</td>
<td>T</td>
<td>OR, 1.77 (1.17-2.68)</td>
<td>.006</td>
<td>30644648</td>
</tr>
<tr>
<td>rs7037276 (IL33)</td>
<td>OR, 0.55 (0.33-0.90)</td>
<td>.02</td>
<td>50</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs1342326 (IL33)</td>
<td>Within 72 h for one-off events after presenting to the ED</td>
<td>C</td>
<td>OR, 0.52 (0.32-0.86)</td>
<td>.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs16986718 (NLRP4)</td>
<td>Number of ED visits/Hosp/OCS/return visit bronchodiolitis/increase in asthma medication</td>
<td>G</td>
<td>Increased annual exacerbation episodes</td>
<td>.001</td>
<td>30526007</td>
<td></td>
</tr>
<tr>
<td>rs1799768 (SERPINE1)</td>
<td>Number of ED visits/Hosp/OCS/return visit bronchodiolitis/increase in asthma medication</td>
<td>G</td>
<td>Increased annual exacerbation episodes</td>
<td>.001</td>
<td>30526007</td>
<td></td>
</tr>
<tr>
<td>rs1799768 (SERPINE1)</td>
<td>Soy isoflavones intake×SNP on OCS</td>
<td>4G/4G/4G5G vs 5G/5G</td>
<td>IRR, 2.57 (1.09-6.07)</td>
<td>.031</td>
<td>30707970</td>
<td></td>
</tr>
<tr>
<td>rs1544410 (VDR)</td>
<td>Exacerbation severity Z-scores TT vs CC</td>
<td>Increased severity scores</td>
<td>.005</td>
<td>32380236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs2228570 (VDR)</td>
<td>Exacerbation severity Z-scores AA vs GG</td>
<td>Increased severity scores</td>
<td>.001</td>
<td>32380236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADRB2 variants</td>
<td>Exacerbation severity Z-scores AG vs GG</td>
<td>Increased severity scores</td>
<td>.011</td>
<td>32380236</td>
<td></td>
<td></td>
</tr>
<tr>
<td>rs67622929 (DNASE1L3)</td>
<td>Discovery: 1002 African American individuals with asthma. Replication: 2181 Hispanic/Latino children with asthma</td>
<td>Hosp/ED visits/OCS in the last 6-12 mo despite ICS plus LABA use</td>
<td>Arg16/Gln27 vs Gly16/Glu27</td>
<td>1.40 (1.05-1.87)</td>
<td>.022</td>
<td>34128573</td>
</tr>
<tr>
<td>rs11681246 (LTBP1)</td>
<td>Hosp/ED visits/OCS in the last 12 mo</td>
<td>C</td>
<td>OR, 1.48 (1.18-1.87)</td>
<td>.03</td>
<td>33035569</td>
<td></td>
</tr>
<tr>
<td>rs76390075 (LTBP1)</td>
<td>Hosp/ED visits/OCS in the last 6-12 mo despite ICS use</td>
<td>G</td>
<td>OR, 0.72 (0.63-0.83)</td>
<td>3.28×10^-4</td>
<td>32786158</td>
<td></td>
</tr>
</tbody>
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(continued)
<table>
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<tr>
<th>rsID (Gene)</th>
<th>Participants</th>
<th>Phenotype</th>
<th>EA/EG</th>
<th>Effect size (95%CI) or (SE)</th>
<th>P Value</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2660845 (LTA4H)</td>
<td>Patients with asthma. Discovery: 523 European individuals with early-onset asthma R1: 2514 European individuals with early-onset asthma R2: 486 Hispanic/Latino children R3: 71 African American children</td>
<td>Hosp/ED visits/OCS in the last 6-12 mo despite montelukast use</td>
<td>G</td>
<td>OR_{Discovery}, 2.92 (1.04-8.18) OR_{R1}, 1.02 (0.87-1.19) OR_{R2}, 1.04 (0.78-1.39) OR_{R3}, 0.27 (0.09-0.80)</td>
<td>.041</td>
<td>34550981 [58]</td>
</tr>
<tr>
<td>rs2517955 (PGAP3)</td>
<td>3-y longitudinal study of 273 non-Hispanic White adolescents and adults with asthma</td>
<td>Number of asthma-related ED visits/Hosp in 3 y</td>
<td>C</td>
<td>β, 1.05 (NA)</td>
<td>.0034</td>
<td>32795586</td>
</tr>
<tr>
<td>rs1341828 (IL1RL1)</td>
<td>2412 European, Hispanic/Latino, or African American children</td>
<td>Hosp/ED visits in the last 6-12 mo despite ICS use</td>
<td>C</td>
<td>OR, 1.32 (1.08-1.62) OR, 1.31 (1.07-1.59)</td>
<td>.02</td>
<td>31755552 [60]</td>
</tr>
<tr>
<td>rs242941 (CRHR1)</td>
<td>European adult patients with asthma (n_{Discovery}: 597; n_{Replication}: 9842)</td>
<td>Hosp/ED visits/OCS despite ICS use</td>
<td>A</td>
<td>RR_{Discovery}, 6.11 (NA) RR_{Replication}, 1.16 (NA)</td>
<td>&lt;.005; .004</td>
<td>33428814 [61]</td>
</tr>
<tr>
<td>rs134481 (TBXT)</td>
<td>1-y longitudinal study of 1454 Korean individuals with asthma, including 955 never-smokers</td>
<td>Number of ED visits/Hosp/OCS/rescue bronchodilator/increase of asthma medication ≥2 exacerbation events vs one/none</td>
<td>C</td>
<td>Increased annual exacerbation episodes OR, 0.36 (0.18-0.72)</td>
<td>.004</td>
<td>34983467 [62]</td>
</tr>
<tr>
<td>rs37973 (GLCCI1)</td>
<td>5710 European adults, 166 European children, 854 Hispanic/Latino children and 493 African American children with asthma</td>
<td>Hosp/ED visits/OCS in the last 6-12 mo despite ICS use/8% decrease in FEV1 in patients after 6 wk of ICS therapy</td>
<td>A</td>
<td>OR, 0.81 (NA)</td>
<td>3.77×10^-4</td>
<td>34971648 [63]</td>
</tr>
</tbody>
</table>

Abbreviations: β, regression coefficient; EA/EG, effect allele/genotype; ED, emergency department; FEV1, forced expiratory volume in the first second; Hosp, hospitalization; ICS, inhaled corticosteroids; IRR, incidence risk ratio; LABA, long-acting β2-agonists; NA, not available; OCS, oral corticosteroid use; OR, odds ratio; RR, relative risk; Rn, replication study (number n); RSV, respiratory syncytial virus; SE, standard error of the β coefficient; SNP, single-nucleotide polymorphism.

*A total of 35 SNPs in linkage disequilibrium (r²≥0.8) were significantly associated with asthma exacerbations on the sample size-weighted meta-analysis based on P values. For visual clarity, the most significant variant in the meta-analysis is shown here, accompanied by the odds ratio corresponding to the largest cohort contributing to the rs9665961 genotype data on the multiancestry meta-analysis.
sequencing, and transcriptomics to develop a multiomics integrative score that prioritized a locus harboring a member of the family of inhibitor of apoptosis proteins (BIRC3) near glucocorticoid receptor–binding sites. The BIRC3 locus was significantly associated with AEs, despite ICS use, in Hispanic/Latino, African American, and European individuals.

**GWAS**

Six nonpharmacogenomic GWAS of AEs were published during the period reviewed (Table 2). An asthma-related HLA-DQB1 locus was associated with AEs in British adults and Hispanic/Latino children, possibly through regulatory effects on HLA genes [71]. In European children, a study comparing participants with AEs and individuals without asthma revealed a novel genome-wide signal in FUT2/MAMSTR, along with several previously asthma-related loci, as expected when such a comparison strategy is applied [74]. Interestingly, the epistasis of a functional FUT2 SNP with an ABO SNP increased the risk of respiratory infection by *Streptococcus pneumoniae* [74].

Most genetic association studies of AEs have been conducted in Europeans [16], although recent substantial efforts have increased ethnic diversity and representation [72,73,75-77]. As a result, the largest GWAS meta-analysis of severe AEs in Hispanic/Latino children (n=4010) uncovered a significant genome-wide association in LINC03033, a long noncoding RNA (lncRNA) that participates in myofibroblast differentiation and airway remodeling [72]. The risk allele for AEs was associated with higher DNA methylation (DNAm) levels in LINC03033 in nasal epithelium. This, in turn, was associated with higher expression of KCNJ2-ASI [72], which is also overexpressed in atopic asthma [84]. Another recent GWAS compared asthma cases with AEs to controls without asthma to overcome the reduced statistical power resulting from the complex genetic structure in recently admixed populations; the objective of the study was to identify genetic signals for asthma in Hispanic/Latino and African American children with AEs [73]. A genome-wide significant locus near lncRNA LINC01913 was associated with asthma with severe exacerbations, possibly through expression of LINC01913 in lung and DNAm of PKDCC in blood. While LINC01913 function remains unknown, PKDCC is involved in lung development and mediates various hostecostatic cellular processes [73]. Likewise, an intrinsic variant in the MYT1L gene, which codes for a regulator of proteins of the nervous system, was associated with the annual number of AEs in Koreans [75]. More recently, a multiancestry meta-analysis of GWAS of AEs identified 2 suggestive variants associated with blood DNAm or expression levels of genes participating in inflammation and host defense (VCAM1, EXT12, and PANK1) [76].

Pharmacogenomic GWAS of AEs have identified susceptibility variants for response to ICS [1,77,80,82,85] and LABAs [79]. Genomic regions harboring the loci suggestively associated with AEs in children receiving ICS are implicated in response to viral infections (APOBEC3B/APOBEC3C [77]), baseline lung function (CACA2D3 [86]), bronchodilator responsiveness (CACA2D3 [87]), and the Wingless/integrase 1 signaling (WT5A) pathway [80]. Moreover, 2 studies considered AEs despite ICS use as a secondary outcome to validate genetic associations for response to ICS at EDDM3B [78] and ROBO2 [81]. While the EDDM3B and APOBEC3B/APOBEC3C variants exerted similar effects across several ethnic backgrounds [77,78], CACA2D3/WT5A and ROBO2 loci exerted specific effects in children of European descent [80,81].

In addition, a recent study in older adults of European ancestry with asthma treated with ICS uncovered 152 suggestive associations for AEs defined using diagnostic codes, and a genome-wide signal for oral corticosteroid (OCS) use near PTCHD4, which encodes a regulator of hedgehog signaling previously associated with airway disease [82]. Furthermore, a genome-wide interaction study of the association between age and AEs despite ICS use found significant genome-wide signals in genes implicated in angiogenesis, lung function, and chronic obstructive pulmonary disease (COPD) (THSD4), inflammatory and immune processes, and response to corticosteroids (HIVEP2) [83]. Moreover, the only multiancestry meta-analysis of GWAS of AEs despite treatment with LABAs revealed suggestive associations within genes previously implicated in lung function (TBX3 [88]) and response to short-acting ß2-agonists (EPHA7 [89]).

As previously indicated, some studies aimed to shed light on the role of genetic variation by assessing their functional and biological impact. For instance, CACA2D3/WT5A and ROBO2 variants were associated with the expression of proteins involved in asthma pathophysiology in plasma [80,81]. Gene-level analysis stratified by smoking status in Koreans revealed that significant genes in nonsmokers were enriched for T-cell immune responses and DNA/RNA modifications, while tissue development and apoptosis were the most important processes in smokers [75].

Genetic variants associated with AEs despite ICS use in European adults are enriched in genes implicated in protein and fatty acid metabolism, toll-like receptor signaling, antigen cross-presentation, and vesicular transport [82]. Among European children treated with ICS, genetic variants associated with AEs were enriched in asthma-related genes that showed differential expression when exposed to trichostatin A [80]. Trichostatin A is an antifungal antibiotic with histone deacetylase activity that has been shown to reduce airway inflammation and hyperresponsiveness [90]. Interestingly, histone deacetylase participates in the regulation of corticosteroid sensitivity [91]. Overall, these findings support the need for further research into the therapeutic potential of trichostatin A in asthma.

**Admixture mapping**

Admixture mapping studies have identified genetic variants associated with asthma, IgE levels, bronchodilator response, and lung function [42,94], although only 2 admixture studies of AEs have been published (Table 3). The only admixture mapping of AEs independent of treatment conducted in Hispanic/Latino individuals revealed significant associations for AEs with Indigenous American ancestry at chromosomal regions 5q32, 13q13-13q2.2, and 3p13. The 5q32 SNP rs1144986 (C5orf46) was significantly and consistently associated with AEs in Mexican Americans and Puerto Ricans but was not validated in non–Hispanic/Latino individuals. The risk allele
<table>
<thead>
<tr>
<th>Type of study</th>
<th>rsID (Gene)</th>
<th>Participants</th>
<th>Phenotype</th>
<th>EA</th>
<th>Effect size</th>
<th>P Value</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>GWAS</td>
<td>rs56151658</td>
<td>Discovery: 34 167 White British adults with asthma; Replication: 2645 Hispanic/Latino children with asthma</td>
<td>ED/Hosp/OCS</td>
<td>A</td>
<td>OR&lt;sub&gt;Discovery&lt;/sub&gt;: 1.36 (1.22-1.52) OR&lt;sub&gt;Replication&lt;/sub&gt;: 1.19 (0.99-1.42)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.11×10&lt;sup&gt;-8&lt;/sup&gt;</td>
<td>32890573 [71]</td>
</tr>
<tr>
<td>GWAS</td>
<td>rs2253681</td>
<td>4010 Hispanic/Latino adolescents with asthma</td>
<td>ED/Hosp/OCS</td>
<td>A</td>
<td>OR: 1.55 (1.34-1.79)</td>
<td>6.3×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>33093117 [72]</td>
</tr>
<tr>
<td>GWAS</td>
<td>rs4952375</td>
<td>Children with asthma. Discovery: 3310 Hispanic/Latino; replication: 1043 African American</td>
<td>ED/Hosp/OCS</td>
<td>A</td>
<td>OR&lt;sub&gt;Discovery&lt;/sub&gt;: 1.37 (1.20-1.55) OR&lt;sub&gt;Replication&lt;/sub&gt;: 1.53 (1.12-2.08)</td>
<td>1.24×10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>32841424 [73]</td>
</tr>
<tr>
<td>GWAS</td>
<td>rs721992318</td>
<td>Discovery: 2866 European children experiencing severe AE between ages 2 and 6 years, and 65 415 nonasthmatic controls. Replication: 1118 children</td>
<td>Discovery: Asthma with Hosp; Replication: Asthma</td>
<td>T</td>
<td>OR: 1.65 (1.56-1.75) OR: 1.41 (1.32-1.51)</td>
<td>1.6×10&lt;sup&gt;-6&lt;/sup&gt;</td>
<td>33328473 [74]</td>
</tr>
<tr>
<td>GWAS</td>
<td>rs10519519</td>
<td>1-year longitudinal study of 20 nonsmoking and 188 smoking Korean patients with asthma</td>
<td>Annual rate of episodes of increased dyspnea, wheezing, or coughing with a &gt;20% decrease in FEV&lt;sub&gt;1&lt;/sub&gt;</td>
<td>A</td>
<td>β: 0.60 (0.11)</td>
<td>8.32×10&lt;sup&gt;-5&lt;/sup&gt;</td>
<td>35606283 [75]</td>
</tr>
<tr>
<td>GWAS</td>
<td>rs12091010</td>
<td>Discovery (4989 patients with asthma): 53.1% European, 23.2% Hispanic/Latino, 13.3% Singaporean Chinese, and 10.3% African American. Replication: 36 477 European and 1078 non-European asthma patients</td>
<td>ACC/ED/Hosp/OCS/SA</td>
<td>T</td>
<td>OR&lt;sub&gt;Discovery&lt;/sub&gt;: 0.82 (0.75-0.90) OR&lt;sub&gt;Replication&lt;/sub&gt;: 0.89 (0.82-0.97)</td>
<td>9.05×10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>35754128 [76]</td>
</tr>
<tr>
<td>PGWAS</td>
<td>rs5995653</td>
<td>Children with asthma. Discovery: 854 Hispanic/Latino, 493 African American. Replication: 1697 European</td>
<td>ED/Hosp/OCS despite ICS use</td>
<td>C</td>
<td>OR&lt;sub&gt;Discovery&lt;/sub&gt;: 0.85 (0.78-0.92) OR&lt;sub&gt;Replication&lt;/sub&gt;: 0.92 (0.86-0.98)</td>
<td>3.10×10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>30697902 [77]</td>
</tr>
<tr>
<td>PGWAS</td>
<td>rs62081416</td>
<td>Children with asthma. Discovery: 854 Hispanic/Latino and 493 African American. Replication: 1697 European</td>
<td>ED/Hosp/OCS despite ICS use</td>
<td>A</td>
<td>OR&lt;sub&gt;Discovery&lt;/sub&gt;: 0.76 (0.62-0.93) OR&lt;sub&gt;Replication&lt;/sub&gt;: 0.66 (0.56-0.79)</td>
<td>4.80×10&lt;sup&gt;-4&lt;/sup&gt;</td>
<td>7.52×10&lt;sup&gt;-3&lt;/sup&gt;</td>
</tr>
<tr>
<td>PGWAS</td>
<td>rs3827907</td>
<td>Patients with asthma. Discovery: 244 African American. Replication: African American (nR1=803 and nR2=563) and Latino (nR3=1461)</td>
<td>Discovery: SNPxICS adherence on change in ACT score over 6 wk of ICS treatment. Replication 1: SNPxICS adherence on time to ED/Hosp/OCS. Replication 2-3: SNPxICS use on ED/Hosp/OCS</td>
<td>C</td>
<td>Coef&lt;sub&gt;SNPxICS&lt;/sub&gt;: 12.35 (NA) Coef&lt;sub&gt;ICStime&lt;/sub&gt;: -0.07 (NA) Coef&lt;sub&gt;ICScnt&lt;/sub&gt;: 0.15 (NA) Coef&lt;sub&gt;ICScnt&lt;/sub&gt;: 0.96 (NA)</td>
<td>7.79×10&lt;sup&gt;-10&lt;/sup&gt;</td>
<td>30367910 [78]</td>
</tr>
</tbody>
</table>

(continued)
### Table 2. Main Findings and Characteristics of Genome-Wide Approaches to Study the Genetic Factors Involved in Asthma Exacerbations Conducted From November 15, 2018 to October 1, 2022 (continuation)

<table>
<thead>
<tr>
<th>Type of study</th>
<th>rsID (Gene)</th>
<th>Participants</th>
<th>Phenotype</th>
<th>EA</th>
<th>Effect size</th>
<th>P Value</th>
<th>PMID</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGWAS (LABA)</td>
<td>rs1947048 (EPHA7)</td>
<td>1425 children and young adults with asthma (23% Hispanic/Latino, 10.4% African American, 32.5 Singaporean Chinese)</td>
<td>ED/Hosp/OCS despite LABA use</td>
<td>G A</td>
<td>OR: 2.50 (1.69-3.69) OR: 1.77 (1.40-2.23)</td>
<td>4.36×10⁻⁶</td>
<td>33706416</td>
</tr>
<tr>
<td></td>
<td>rs6489992 (TBX3)</td>
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<td>[79]</td>
</tr>
<tr>
<td>PGWAS (LABA)</td>
<td>rs67026078 (C4NA2D3/WNT5A)</td>
<td>Children with asthma. Discovery: 2681 European. Replication 2: 854 Hispanic/Latino, 493 African American, 426 Singaporean Chinese</td>
<td>ED/Hosp/OCS/SA despite ICS use</td>
<td>C</td>
<td>ORDiscovery: 1.50 (0.93-2.43) ORReplication: ROR1: 1.83</td>
<td>4.22×10⁻⁶</td>
<td>33303529</td>
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<td>[80]</td>
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<tr>
<td>PGWAS (ICS)</td>
<td>rs1166980 (ROBO2)</td>
<td>Children with asthma. Discovery: 166 European. Replication 1: 2681 European. Replication 2: 854 Hispanic/Latino, 493 African American</td>
<td>Discovery: ≥8% in FEV1 after 6 wk of ICS treatment</td>
<td>G</td>
<td>ORDiscovery: 7.01(2.39-14.93)</td>
<td>4.61×10⁻³</td>
<td>34442380</td>
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<td>[81]</td>
</tr>
<tr>
<td>PGWAS (ICS)</td>
<td>rs72891545 (ROBO2)</td>
<td>European adults with asthma (rs56151658 was not available in Hispanic/Latino individuals. The results for the most significant proxy in Latinos (r²=0.71) are shown (rs9275356).)</td>
<td>OCS despite ICS use</td>
<td>G</td>
<td>ORDiscovery: 1.73 (1.39-2.16) ORReplication: 1.48 (0.75-2.90)</td>
<td>7.91×10⁻⁷</td>
<td>35501119</td>
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<td>[82]</td>
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<tr>
<td>PGWAS (ICS)</td>
<td>rs138717703 (RBX1P1/PTCHD4)</td>
<td>European adults with asthma (rs1144986 was associated with altered DPYSL3 DNAm levels and lower gene expression of SCGB3A2 in blood. While DPYSL3 may be involved in airway remodeling, SCGB3A2 is an upstream regulator of TGFβ-mediated antifibrotic processes in the lung [92].)</td>
<td>OCS despite ICS use</td>
<td>C</td>
<td>ORDiscovery: 1.73 (1.39-2.16) ORReplication: 1.48 (0.75-2.90)</td>
<td>7.91×10⁻³</td>
<td>7.91×10⁻³</td>
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<td>[82]</td>
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<tr>
<td>PGWAS (ICS)</td>
<td>rs77506063 (RBX1P1/PTCHD4)</td>
<td></td>
<td></td>
<td>C</td>
<td>ORDiscovery: 1.74 (1.40-2.16) ORReplication: 1.27 (0.66-2.42)</td>
<td>6.18×10⁻⁷</td>
<td>3.56×10⁻³</td>
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<td></td>
<td>[82]</td>
</tr>
<tr>
<td>PGWAS (ICS)</td>
<td>rs145325916 (RBX1P1/PTCHD4)</td>
<td></td>
<td></td>
<td>C</td>
<td>ORDiscovery: 1.74 (1.40-2.16) ORReplication: 1.27 (0.66-2.42)</td>
<td>6.18×10⁻⁷</td>
<td>3.56×10⁻³</td>
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<td>[82]</td>
</tr>
<tr>
<td>PGWAS (ICS)</td>
<td>rs116023293 (HNRNPM3/P4 PTCHD4)</td>
<td></td>
<td></td>
<td>G</td>
<td>ORDiscovery: 1.74 (1.40-2.16) ORReplication: 1.27 (0.66-2.42)</td>
<td>5.28×10⁻¹⁰</td>
<td>3.56×10⁻³</td>
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<td>[82]</td>
</tr>
<tr>
<td>PGWIS (Age; ICS)</td>
<td>rs34631960 (THSD4)</td>
<td>1321 European adult and SNPxAge use on children with asthma</td>
<td>ED/Hosp/OCS</td>
<td>C</td>
<td>ORDiscovery: 2.33 (1.61-3.38) ORReplication: 1.82 (1.23-2.73)</td>
<td>7.08×10⁻⁶</td>
<td>32119686</td>
</tr>
<tr>
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<td>[83]</td>
</tr>
<tr>
<td>PGWIS (Age; ICS)</td>
<td>rs2328386 (HIVEP2)</td>
<td>1321 European adult and SNPxAge use on children with asthma</td>
<td>ED/Hosp/OCS</td>
<td>T</td>
<td>ORDiscovery: 0.33 (0.54-0.77) ORReplication: 0.51 (0.34-0.77)</td>
<td>1.86×10⁻¹⁰</td>
<td>1.49×10⁻⁷</td>
</tr>
</tbody>
</table>

Abbreviations: ACC, acute asthma care; ACT, Asthma Control Test; Coef, interaction coefficient estimate; EA, effect allele; ED, emergency department; FEV₁, forced expiratory volume in the first second; GWAS, genome-wide association study; Hosp, hospitalizations; ICS, inhaled corticosteroids; LABA, long-acting β-agonists; OCS, oral corticosteroid use; NA, not available; NS, nonsignificant; PGWAS, pharmacogenomic GWAS (asthma treatment considered is shown within parenthesis); PGWIS, pharmacogenomic genome-wide interaction study (tested environmental variable and asthma treatment considered are shown within parenthesis); Rn, replication study (number n); SA, school absences; SNP, single-nucleotide polymorphism.

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plus salmeterol. Moreover, African ancestry at chromosome 22q12.1 was associated with better responsiveness in adults who transitioned from low-dose ICS to the quintuple dose of ICS than in those who received 2.5 times the ICS dose. Analysis of genetic variants within these regions revealed 1 SNP that was consistently replicated for association with AEs in African Americans treated with ICS [93].

**Epigenetics**

The 3 main epigenetic mechanisms that act synergistically to regulate gene expression are DNAm, histone modifications, and noncoding RNAs such as micro-RNAs (miRNA). However, histone modifications have been investigated for asthma [95] but not for AEs.

**DNAm**

DNAm consists of the addition of a methyl group to a cytosine, often within 5′-cytosine-phosphate-guanine-3′ dinucleotide sequences (or CpG sites). DNAm levels have been associated with disease risk and health outcomes, including asthma and allergy [96]. Most CpGs in the human DNA methylome are hypermethylated and located in regions of low CpG density. However, CpG-rich regions, known as CpG islands, show less DNA methylation and have been associated with gene expression.

### Table 3. Main Findings and Characteristics of the Admixture Mapping Studies of Asthma Exacerbations Conducted From November 15, 2018 to October 1, 2022

<table>
<thead>
<tr>
<th>rsID (Gene)</th>
<th>Chromosomal band</th>
<th>Participants</th>
<th>Phenotype</th>
<th>EA OR (95%CI)</th>
<th>P Value</th>
<th>PMID [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs1144986</td>
<td>5q32</td>
<td>Patients with asthma.</td>
<td>ED/Hosp/OCS</td>
<td>Discovery: 0.43 (0.28-0.66)</td>
<td>9.45x10^{-2}; 4.94x10^{-2}; NS</td>
<td>36180068 [92]</td>
</tr>
<tr>
<td>rs7552429</td>
<td>2q12.1</td>
<td>Discovery: 266 adolescents/adults of African descent</td>
<td>Better response to 5xICS vs 2.5xICS</td>
<td>Discovery: 0.21 (0.09-0.52)</td>
<td>6x10^{-2}; 0.003</td>
<td>34762840 [93]</td>
</tr>
<tr>
<td>rs73399224</td>
<td>12q24.22</td>
<td>Discovery: 250 children of African descent</td>
<td>Better response to 5xICS vs 100 μg fluticasone plus salmeterol</td>
<td>Discovery: 0.17 (0.07-0.42)</td>
<td>8.0x10^{-4}; 0.03</td>
<td></td>
</tr>
</tbody>
</table>

### Table 4. Main Findings of the Studies of DNAm in Asthma Exacerbations

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>Participants</th>
<th>Phenotype</th>
<th>CpG</th>
<th>Gene/Nearest Gene</th>
<th>Regression coefficient (95%CI)</th>
<th>P Value</th>
<th>PMID [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cord blood</td>
<td>303 children recruited by a hospitalization cohort in Manchester (UK)</td>
<td>Asthma-related hospitalizations or ED after the first year of life</td>
<td>cg00066816</td>
<td>IL2</td>
<td>1.07 (1.01,1.14)</td>
<td>.03</td>
<td>23414538 [98]</td>
</tr>
<tr>
<td>Blood</td>
<td>394 children treated with ICS (57.4% European, 42.6% Hispanic/Latino)</td>
<td>Asthma-related hospitalizations or ED in the past year</td>
<td>cg00557354</td>
<td>IL2</td>
<td>−3.10 (NA)</td>
<td>.002</td>
<td>31187518 [99]</td>
</tr>
</tbody>
</table>

Abbreviations: ED, emergency department visits; ICS, inhaled corticosteroids; NA, not available OCS; OCS, oral corticosteroid bursts.
### Table 5. Main Findings and Characteristics of miRNA Studies in the Context of Asthma Exacerbations

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>miRNA profiling</th>
<th>Participants</th>
<th>Phenotype</th>
<th>Main findings</th>
<th>PMID [reference]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole blood</td>
<td>Human MicroRNA v2.0 Assay Pool (Illumina)</td>
<td>Children with (n=100) and without asthma (n=100) recruited at a Turkish hospital</td>
<td>Asthma severity (GINA, 2008) and severity of adverse event</td>
<td>Increased expression of 10 miRNAs was associated with asthma severity and exacerbations severity: HS_108.1, HS_112, HS_182.1, HS_240, HS_261.1, HS_3, HS_55.1, HS_91.1, hsa-miR-604, and hsa-mir-638.</td>
<td>26422695 [104]</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>q-PCR</td>
<td>Children with acute-stage asthma (n=100) and healthy children (n=100) recruited at a Chinese hospital</td>
<td>Acute asthma attacks (not defined)</td>
<td>miR-1 expression levels were reduced in acute-stage asthma compared with controls. miR-1 expression levels improved prediction of acute asthma attacks compared with IL-4, IL-5, IL-8, and TNF-α in the same population.</td>
<td>30046607 [105]</td>
</tr>
<tr>
<td>Serum</td>
<td>q-PCR</td>
<td>Participants with (n=59) and without asthma (n=11) recruited in the United States</td>
<td>Lifetime and past 12 mo frequency of asthma-related hospitalizations</td>
<td>miR-1 levels were inversely correlated with sputum eosinophilia and asthma-related hospitalization frequency, and positively correlated with lung function and ACT scores.</td>
<td>32035607 [106]</td>
</tr>
<tr>
<td>Serum</td>
<td>q-PCR</td>
<td>European children with asthma from CAMP: 38 with and 115 without exacerbations</td>
<td>OCS bursts in the past 12 mo following randomization with ICS</td>
<td>Increased expression of 12 miRNAs was associated with OCS bursts: miR-206, miR-146b-5p, miR-222-3p, miR-409-3p, miR-223-5p, miR-126-5p, miR-339-3p, miR-30e-3p, miR-126-3p, miR-342-3p, miR-454-3p, and miR-720. A clinical and 3-miRNA model (miR-146b, miR-206, and miR-720) showed higher AUC for prediction of OCS use compared with the clinical model in the same population (AUC: 0.81 vs 0.67).</td>
<td>29940952 [107]</td>
</tr>
<tr>
<td>Serum</td>
<td>q-PCR</td>
<td>6-wk longitudinal study of 21 adults with asthma recruited at a Polish hospital</td>
<td>Admission for an unplanned visit due to worsened symptoms of asthma accompanied by a decrease in ventilatory parameters</td>
<td>Reduced levels of miRNA-126a, miRNA-16, and miRNA-21 during the exacerbation compared with the follow-up visit.</td>
<td>31743969 [108]</td>
</tr>
<tr>
<td>Induced sputum</td>
<td>Nanostring nCounter array v3.0a</td>
<td>Participants with (n=62) and without asthma (n=9) recruited in the United States</td>
<td>Asthma-related hospitalizations in the past 12 months</td>
<td>A 12 miRNA. WGCNA module was directly correlated with asthma hospitalizations. Ten of these miRNA correlated significantly and consistently with sputum neutrophils, longer duration of asthma, decreased quality of life, impaired lung function, and/or increased BDR. The miRNA module correlated with a mRNA module enriched in genes participating in TLR/T,17 signaling and endoplasmic reticulum stress.</td>
<td>32255668 [109]</td>
</tr>
<tr>
<td>Whole blood</td>
<td>Small-RNA sequencing</td>
<td>Costa Rican children with asthma (n=183; n0=168) from GARCS</td>
<td>≥3 events of asthma-related ED/AC visits and/or hospitalizations in the last 12 mo (frequent exacerbations, FE) compared with no or infrequent exacerbation (IF)</td>
<td>5 miRNA (miR-451b, hsa-miR-142-5p, hsa-miR-6739-3p, hsa-miR-7-5p, and hsa-miR-4433b-5p) were downregulated in FE compared with IF. 15 miRNA (hsa-miR-93-3p, hsa-miR-766-3p, hsa-miR-331-3p, hsa-miR-532-3p, hsa-miR-664b-3p, hsa-miR-296-5p, hsa-miR-6515-3p, hsa-miR-4286, hsa-miR-1296-5p, hsa-miR-29b-2-5p, hsa-miR-500b-5p, hsa-miR-500a-5p, hsa-miR-642a-5p, hsa-miR-103a-2-5p, and hsa-miR-550a-3p) were upregulated in FE compared with IF. miR-532-3p, miR-296-5p, miR-766-3p, miR-7-5p, and miR-451b also showed significant association with COPD exacerbations.</td>
<td>35447890 [110]</td>
</tr>
</tbody>
</table>

Abbreviations: AC, acute care; AUC, area under the curve; CAMP, Childhood Asthma Management Program; COPD, chronic obstructive pulmonary disease; ED, emergency department; FE, frequent exacerbations; GARCS, Genetics of Asthma in Costa Rica Study; GINA, Global Initiative for Asthma; ICS, inhaled corticosteroids; IF, no or infrequent exacerbations; mRNA, messenger RNA; OCS, oral corticosteroids; q-PCR, real-time quantitative polymerase chain reaction; RNA, ribonucleic acid; T,17, T-helper type-17 cells; TLR, toll-like receptor; WGCNA, weighted gene co-expression network analysis.
islands, are often hypomethylated. While promoter DNA methylation usually leads to reduced gene expression, gene body DNA methylation is associated with active transcription [97].

Although DNA methylation is the most extensively studied epigenetic mechanism, only 1 targeted DNA methylation study of AEs [98] and 1 epigenome-wide association study (EWAS) [99] of AEs as a proxy of treatment response have been published (Table 4). Curtin et al [98] found that increased cord blood DNA methylation of the IL2 promoter was associated with AEs and hospitalizations for asthma or wheezing later in childhood. Furthermore, Wang et al [99] conducted a multiancestry EWAS meta-analysis of peripheral blood CpG markers and AEs despite treatment with inhaled corticosteroids (ICS). Hypomethylation of cg00066816 upstream of the IL12B gene, which encodes for a subunit of the heterodimeric IL-12, a proinflammatory cytokine involved in Th1 and Th17 signaling [100], was nominally associated with the absence of asthma-related ED visits or hospitalizations in the previous year in children taking ICS. In a secondary analysis, 13 CpGs were differentially methylated in patients who received OCS bursts in the previous year despite ICS use. Although functional effects of DNA methylation on blood gene expression were explored, the CpG-gene pairs were not consistently replicated across studies [99].

**miRNAs**

miRNAs are posttranscriptional regulators that exert their effects by binding to the 3′ untranslated regions of mRNAs, leading to mRNA deadenylation and subsequent degradation. These small noncoding molecules are implicated in the regulation of multiple cellular processes and have recently gained attention in allergic and chronic lung diseases [101-103].

To our knowledge, 7 studies have addressed the role of miRNAs in AEs, including 3 studies in blood, 3 in serum, and 1 in induced sputum (Table 5). All studies focusing on circulating miRNAs applied single marker approaches, while the study that performed miRNA profiling in induced sputum applied a systems biology approach. Specifically, Gomez et al [109] conducted a weighted gene coexpression network analysis (WGCNA) of miRNA and mRNA expression levels in induced sputum from 61 individuals with asthma. The analysis of 221 miRNAs revealed a 12-miRNA module directly correlated with asthma hospitalizations. In their cluster analysis, high expression levels of these 12 miRNAs were associated with neutrophilic inflammation, low T2 biomarkers, and airflow obstruction. Notably, the sputum miR-223-3p module correlated with mRNA modules implicated in the TLR9/TLR17 signaling pathway and endoplasmic reticulum stress. One of the miRNAs associated with high sputum neutrophil counts in response to ozone exposure, hsa-miR-223-3p, acted as a regulator of both mRNA modules [109].

Midyat et al [104] reported that 10 of 739 tested miRNAs were differentially expressed according to the severity of asthma and AEs in children. Another study found that miR-1 was downregulated in acute-stage asthma and predicted asthma attacks with an AUC of 0.90, which was significantly higher than the AUC from asthma-related cytokines (eg, IL-4 or IL-5) (P<.05) [105]. Analysis of animal models and primary human endothelial cells has shown miR-1 to be implicated in the regulation of airway eosinophilia through the inhibition of eosinophilic binding to the endothelium by promoting RNA-induced gene silencing of eosinophil trafficking genes [106].

In a 6-week longitudinal study, expression of 3 of 7 circulating miRNAs tested (miRNA-126a, miRNA-16, and miRNA-21) were significantly lower during an AE episode than at a follow-up visit [108]. Furthermore, miRNA-21 and miRNA-126a expression levels were positively correlated with FEV1%, whereas miRNA-21 levels were higher in patients with atopy or FeNO levels >25 ppb. miRNA-126a and miRNA-21 are both considered promoters of Th2-mediated allergic inflammation [101,111], and miRNA-21 is a systemic oxidative stress marker that is dysregulated in the airways and/or blood in atopic dermatitis and allergic asthma [101].

In an analysis of patients who experienced frequent exacerbations and infrequent/no exacerbations, 20 of 649 tested blood miRNAs were differentially expressed by individuals with asthma [110]. In the COPDGene study, 5 of these 20 miRNAs were associated with COPD exacerbations, supporting some overlap in the pathogenesis of COPD and asthma. The gene targets of these 4 miRNAs participate in the PI3K-Akt and MAPK signaling pathways [110], which are relevant to Th2 inflammation and asthma pathogenesis [102].

<table>
<thead>
<tr>
<th>Biological sample</th>
<th>RNA profiling</th>
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<td>Nasal blow (NextSeq 500 platform)</td>
<td>RNA-seq</td>
<td>208 children with asthma from the United States</td>
<td>OCS/ Hospitalization</td>
<td>Increased SMAD3 expression among children with altered abundance of the two bacterial network was associated with increased exacerbation risk.</td>
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<td>Bronchial biopsies</td>
<td>Genome U133 Plus 2.0 Array (Affymetrix)</td>
<td>≥2 events of systemic corticosteroids use vs FE &lt;2 events IE</td>
<td>CEACAM5 expression was increased in FE compared with IE. Higher expression scores for viral infection gene signatures, type 1, T-helper type-17, and type 2 activation pathways in FE compared to IE. Higher expression scores of type 2, type 1 and steroid insensitivity pathway signatures in persistent FE compared to persistent IE.</td>
<td>35474304 [116]</td>
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Abbreviations: FE, frequent exacerbators; IE, infrequent exacerbators; OCS, oral corticosteroids use; RNA-seq, RNA sequencing.
Only 1 study has investigated the role of miRNAs in response to treatment using AEs as a clinical endpoint. In particular, miRNA profiling of serum samples was conducted in 153 children with asthma after randomization to ICS [107]. The univariate logistic regression models showed 12 of the 125 tested serum miRNAs to be significantly associated with OCS bursts in the previous year. Moreover, the combination of a clinical score for exacerbations along with 3 of these 12 miRNAs (miR-146b, miR-206, and miR-720) suggested a higher predictive capability for AEs than clinical score alone (AUC, 0.81 vs 0.67) [107]. Of note, in individuals with asthma, serum miR-146b-5b and miR-206 levels have been associated with asthma and COPD [103], as well as with baseline FEV/FVC [112].

Transcriptomics

Studies of transcriptomics and AEs prior to 2022 were recently reviewed by Gautam et al [37]. The authors identified distinct AE-related gene expression signatures implicated in innate and adaptive immunity and in viral and nonviral exacerbations and revealed genes implicated in frequent exacerbations (TNFR2) and in AEs triggered by cold (genes implicated in the SMAD3 signaling pathways). The only single-cell RNA-sequencing study conducted in the context of AEs highlighted the implication of several cytokines and intracellular transduction regulators in multiple cell types in these asthma outcomes [113].

Two transcriptomic studies of AEs were published during the period reviewed (Table 6). One focused on the effect of the interaction between transcriptional and bacterial networks in nasal epithelium on the risk of AEs in children [114]. Specifically, the risk of AEs increased along with the expression of genes implicated in SMAD3-related cell differentiation in a context of high abundance of a bacterial network dominated by Veillonella, Streptococcus, Neisseria, and Haemophilus and/or reduced abundance of a bacterial network dominated Staphylococcus [114]. The other aimed to understand the pathophysiological factors underlying frequent exacerbations using transcriptomic data from bronchial biopsies. CEACAM5, which encodes a cell surface glycoprotein upregulated by interferon-γ [115], was the only transcript differentially expressed in individuals with frequent exacerbations compared to those with infrequent exacerbations. However, no differential expression was found when individuals with persistent frequent exacerbations were compared with those with persistent infrequent exacerbations. An analysis of several gene signatures for viral infections and type 1 and type 2 inflammatory pathways revealed that individuals with frequent exacerbations showed higher expression of those signatures than individuals with persistent frequent exacerbations [116].

Conclusion and Future Directions

AEs constitute a major burden for individuals with asthma and their caregivers, health care systems, and society as a whole. Although preventing AEs is key in clinical practice, stratification of patients with AEs by risk is challenging owing to the inherent heterogeneity of the biological mechanisms underlying these events. Nevertheless, -omics studies have identified genes and biological processes associated with AEs and proposed potential therapeutic targets. These results need to be validated in independent cohorts and experimental studies, and much work remains to be done in terms of comparison with -omics findings in other respiratory traits, such as asthma [37] and COPD [117-119].

Given the heterogeneity of AEs, specific phenotyping approaches may be successful in identifying novel susceptibility variants [58,74,120]. Despite the increased statistical power derived from a large sample size, future studies should also consider analyzing subtypes or homogenous groups of individuals exposed to similar exacerbation triggers. These would enable the characterization of gene–environment interactions, which are almost unexplored in AEs [1]. An alternative approach to boost statistical power in recently admixed populations is to leverage local ancestry into GWAS models to increase the resolution of causal variant identification [121]. Interestingly, differences in the definition of AEs, triggers, and clinical characteristics of individuals with asthma may have reduced statistical power in several GWAS [76,77,79,80,122]. This could also account, at least partially, for the lack of replication of SNPs associated with AEs across independent populations [76].

A combination of genetic variants into a single risk burden score or polygenic risk score (PRS) for AEs is not feasible without additional risk stratification that also considers clinical and environmental parameters. Recently, a multiancestral PRS for asthma developed using lasso [123] or Bayesian regression [124] captured the risk of asthma, although other studies have failed to achieve this [125,126]. A PRS incorporating DNAm or gene expression data may better capture environmental influences and improve risk stratification [127]. The extent to which methylation risk scores or transcriptome risk scores may contribute to risk prediction remains to be determined, although promising findings have been published for other respiratory traits [128,129]. Within this context, it will be crucial to evaluate the predictive power of biomarkers in populations not included in the discovery phase or training datasets [130].

Similarly, the severity and number of AEs have a prognostic capability in risk stratification [131], although only 1 GWAS of the annual number of exacerbations has been conducted [75], and no studies have assessed the temporal distance between events and/or the time to first exacerbation. Moreover, although bioinformatics tools have been used to evaluate the functional impact of potential susceptibility variants, many of these resources do not include data from tissues/cells obtained from individuals with asthma, across several asthma-relevant tissues, or from diverse ethnic backgrounds. In this sense, experimental studies are required if we are to understand the biological role of the genes identified and establish their prognostic value with the aim of adequately implementing precision medicine in patient risk stratification and prioritizing potential therapeutic targets.

The role of rare variants in AEs has been poorly investigated, even though they may underlie ethnic/racial differences in
the burden of AEs or interact with environmental exposures to modulate AEs. Furthermore, although ethnic diversity has increased in genetic studies of asthma, particularly for Hispanic/Latino populations, large-scale genome-wide studies of populations of Asian and African descent have not been implemented.

The contribution of the DNA methylome to AEs remains largely unexplored [99]. Thus, a priority in asthma epigenetics is to investigate the role of DNAm as a mediator of environmental effects or as a consequence of AEs, not only at the CpG level, but also in differentially methylated regions. Future research should also focus on the role of genetically regulated DNAm and epigenetically regulated gene expression. Furthermore, it will be key to consider that hypomethylation states in previous EWAS of asthma in blood were largely driven by a lower eosinophil count in blood [132], which is why interest is growing in specific cell types [132,133] and cell-type deconvolution algorithms to discern cell-type specific DNAm signals using whole-blood data [134]. Moreover, histone modifications have been implicated in asthma susceptibility and severity, response to ICS, and immune responses to viral infections [95,135], yet little is known about histone modifications and AEs.

Most epigenetic asthma studies have focused on miRNAs in blood and serum, and their findings must be validated to exclude spurious results due to differences in sample processing [136,137]. Despite this concern, many miRNAs have been consistently implicated in chronic respiratory and allergic diseases, highlighting their potential as possible therapeutic targets (eg, miR-206 and miRNA-21) [101-103]. Other plausible candidates in AEs are miRNAs involved in airway inflammation and respiratory infections [101,102,138]. Undoubtedly, further exploration of the role of the miRNAome and its interaction with other -omics layers in the upper and lower airways is required to determine the role of altered miRNA expression in AEs.

Transcriptomic studies conducted during the period reviewed highlight important host-microbiome interactions in the upper and lower airways and open new directions for future research. Although McCauley et al [114] found that the interaction between host gene expression levels and microbial networks in the upper airways promotes AEs, the causative direction of those relationships remains unclear. Still, the authors proposed several plausible candidate genes that could be evaluated in other cohorts. Interestingly, among individuals with frequent AEs, Hoda et al [116] found increased CEACAM5 expression, which is also promoted by interferon γ [115].

Despite recent progress in genomic studies of AEs, the role of and interaction between different -omics layers in the modulation of the risk of AEs remain largely unexplored. In many cases, novel findings have yet to be validated in independent populations, and their prognostic potential is unclear. Key future determinants for identification of accurate biomarkers of AEs for precision medicine will include multiethnic cohorts with better phenotyping of clinical and environmental characteristics, careful phenotyping approaches, evaluation of longitudinal exacerbation data, and combination or integration of different -omics layers of data.

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Conflicts of Interest

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