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REVIEW ARTICLE

Decoding the genetic and epigenetic basis of asthma

Bernard S. Stikker[1] | Rudi W. Hendriks[1] | Ralph Stadhouders[1,2]

[1]Department of Pulmonary Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

[2]Department of Cell Biology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands

Correspondence
Rudi W. Hendriks and Ralph Stadhouders, Department of Pulmonary Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands.
Email: r.hendriks@erasmusmc.nl and r.stadhouders@erasmusmc.nl

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Abstract

Asthma is a complex and heterogeneous chronic inflammatory disease of the airways. Alongside environmental factors, asthma susceptibility is strongly influenced by genetics. Given its high prevalence and our incomplete understanding of the mechanisms underlying disease susceptibility, asthma is frequently studied in genome-wide association studies (GWAS), which have identified thousands of genetic variants associated with asthma development. Virtually all these genetic variants reside in non-coding genomic regions, which has obscured the functional impact of asthma-associated variants and their translation into disease-relevant mechanisms. Recent advances in genomics technology and epigenetics now offer methods to link genetic variants to gene regulatory elements embedded within non-coding regions, which have started to unravel the molecular mechanisms underlying the complex (epi)genetics of asthma. Here, we provide an integrated overview of (epi)genetic variants associated with asthma, focusing on efforts to link these disease associations to biological insight into asthma pathophysiology using state-of-the-art genomics methodology. Finally, we provide a perspective as to how decoding the genetic and epigenetic basis of asthma has the potential to transform clinical management of asthma and to predict the risk of asthma development.

KEYWORDS
asthma, epigenetics, EWAS, GWAS, PRS

1 | INTRODUCTION

Asthma is a common and heterogeneous non-communicable disease that affects over 300 million people worldwide. Asthma is characterized by variable respiratory symptoms such as coughing, wheezing, chest tightness, and airflow limitation caused by chronic airway inflammation, tissue remodeling, bronchial hyperresponsiveness, and mucus hypersecretion.[1–3] Global asthma prevalence is increasing rapidly, and despite the significant advances in asthma treatment over the past decades, approximately 10% of asthma patients do not adequately respond to standard therapy.[4]

Why susceptible individuals develop asthma whereas others do not is a central question in the field that remains only partially resolved to date. Environmental factors early in life, such as viral infections or allergen exposure, provide an important piece to this complex puzzle.[5] In addition, genetics plays a critical role in explaining asthma susceptibility. Over the past 12 years, genome-wide association studies (GWAS) have made tremendous strides in annotating this genetic component of asthma susceptibility.[6] The vast majority of the identified genetic variants are not associated with altered protein function but are instead enriched in non-coding gene regulatory elements (GREs)[7,8]–small genomic regions that can control gene

expression.[9] GREs, such as promoters and enhancers, are bound by DNA-binding proteins called transcription factors (TFs) that regulate gene transcription. TF action is heavily influenced by epigenetic modifications of the genome—collectively referred to as the epigenome.[10] These include DNA methylation and post-translational histone modifications, which affect the ability of TFs to bind DNA and control gene expression.[11] Since DNA methylation is a heritable modification,[12] epigenome-wide association studies (EWAS) have searched for DNA methylation changes associated with the development of asthma.[13,14] By modulating gene regulatory processes, genetic, and epigenetic variants can affect gene expression levels and as a consequence cellular function.

Despite the wealth of potentially disease-relevant information provided by GWAS and EWAS, follow-up studies have been relatively scarce due to the complexity with which (epi)genetic variants may impact gene regulation in different cell types. Fortunately, recent advances in epigenomics technology now provide a toolbox for linking disease-associated variants to transcriptional control mechanisms. This review provides a detailed overview of the (epi)genetic landscape linked to asthma susceptibility, focusing specifically on recent findings from GWAS and EWAS. Importantly, we provide an update on the latest developments in epigenomic approaches to further decode the (epi)genetic basis of complex diseases such as asthma, which offers promising opportunities to move our knowledge on the (epi)genetics of asthma toward clinically relevant applications.

2 | THE PATHOPHYSIOLOGY OF ASTHMA

Traditionally, asthma has been grouped into two broad subtypes: allergic and non-allergic asthma. However, this classification turned out to be an oversimplification,[15] as extensive inflammatory and clinical heterogeneity exists among asthma patients that is not captured by the allergic/non-allergic dichotomy.[2,4] More recently, the field has adopted a distinction based on the presence ("T2-high") or absence ("T2-low") of substantial type-2 inflammation in the airways.[3,16] T2-high asthma mainly involves allergic disease types and affects most children with asthma as well as approximately 50% of adult patients. The chronic type-2 immune response in T2-high asthma, including allergic asthma, is characterized by an eosinophilic airway inflammation. In allergic patients, the disease starts with sensitization to inhaled allergens such as house dust mite (HDM), animal dander, pollen, or fungal spores during childhood that eventually results in chronic airway inflammation. Allergic patients can be defined by the presence of immunoglobulin E (IgE) antibodies in the serum and/or a positive skin prick test for inhaled allergens. In contrast, patients with T2-low asthma are usually non-allergic and often have a more neutrophilic or paucigranulocytic inflammatory profile.[2,3]

In individuals susceptible for developing allergic T2-high asthma, a type-2 immune response is initiated by lung epithelial cells that release the alarmin cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) upon allergen exposure (Figure 1). Alarmins stimulate dendritic cells (DCs) to take up allergens and migrate to the lymph

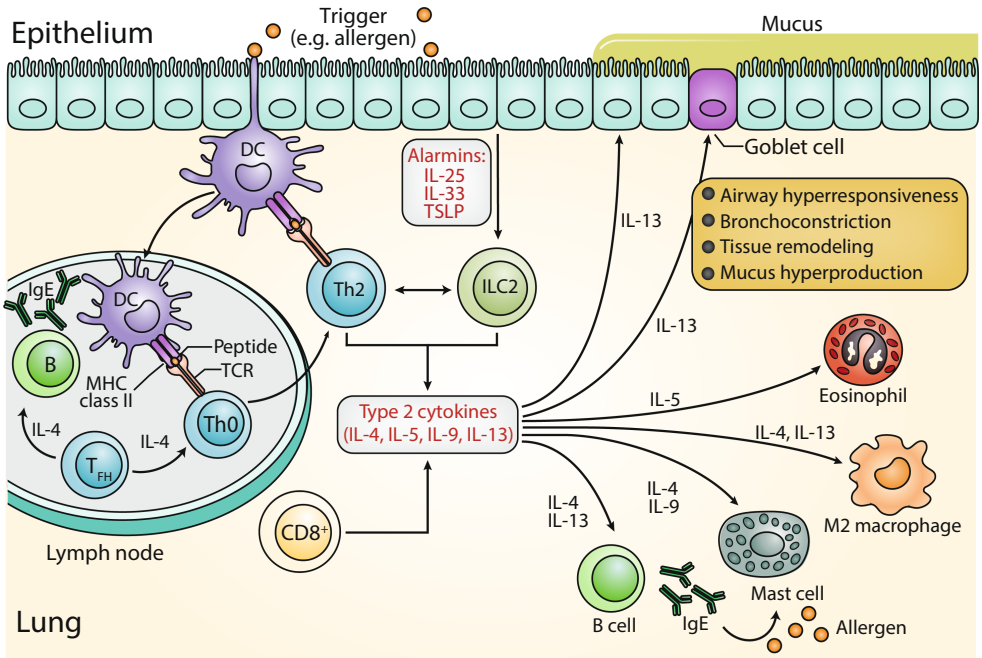


FIGURE 1 Pathophysiology of type-2 asthma. In susceptible individuals, allergens induce the airway epithelium to produce and release alarmin cytokines IL-25, IL-33, and thymic stromal lymphopoietin (TSLP). These activate dendritic cells (DC) to migrate toward the lymph node and present allergen-derived antigens to naive T cells (T_H0). IL-4 produced by T follicular helper (T_{FH}) cells stimulates naive T cells to differentiate towards T_H2 cells, which migrate to the lung. In addition, IL-4 produced in the lymph node by the T_{FH} cells initiates class switching of B cells to produce IgE antibodies. In the lung, not only T_H2 cells but also type 2 innate lymphoid cells (ILC2s) and CD8+ T cells produce type 2 cytokines, which subsequently instigate effector functions in B cells, M2 Macrophages, mast cells, and eosinophils. Finally, the chronic type-2 inflammation induces hallmark asthma symptoms (listed in the orange box).[23] Adapted with permissions form: Walker, J., McKenzie, A. TH2 cell development and function. Nat Rev Immunol 18, 121–133 (2018). https://doi.org/10.1038/nri.2017.118.

node, where they present them to naïve CD4$^+$ T cells. Combined with exposure to IL-4 produced by follicular T helper (T_{FH}) cells, these naive cells will differentiate into T helper 2 (T_H2) effector cells that migrate toward the lung.[3,17,18] Furthermore, IL-4 produced by T_{FH} cells induces IgE class switching in B cells in the lymph node,[19] as well as locally in the lung in inducible bronchus-associated lymphoid tissue (iBALT).[20] IgE produced by class-switched plasma cells activates pulmonary mast cells by antigen-mediated crosslinking of their high-affinity Fc receptor for IgE (FcεRI), to release pro-inflammatory mediators such as histamine, leading to smooth muscle contraction and airway mucosal edema.[21] Of note, (allergic) T2-high asthma is frequently accompanied by a parallel chronic inflammation occurring in the upper airways (i.e. rhinitis, rhinosinusitis), which has led to the united airway disease concept.[22]

In the lung, T_H2 cells produce the canonical type 2 cytokines IL-4, IL-5, IL-9, and IL-13, which can explain all hallmarks of T2-high asthma. IL-5 stimulates the development of eosinophils in the bone marrow and their recruitment to the lung. IL-9 promotes mast cells survival and proliferation, goblet cell metaplasia, and airway wall remodeling, whereas IL-13 acts directly on epithelial integrity by impairing tight junctions and inducing bronchial hyperreactivity (BHR) and mucus hyperproduction.[2,4,24,25] In addition to T_H2 cells, other cell types can produce type 2 cytokines, including CD8$^+$ T cells,[26] mast cells,[27] basophils[28] and in particular group 2 innate lymphoid cells (ILC2).[29,30] ILC2s are innate counterparts of T_H2 cells, which lack an antigen-specific receptor but respond to alarmins by producing copious amounts of type 2 cytokines.[31]

T2-low asthma does not feature a prominent type-2 immune response and is associated with a late onset of disease, poor response to corticosteroid therapy, and obesity.[3] Clinical heterogeneity and a lack of proper mouse models have resulted in a poor understanding of the immunological basis of T2-low asthma. The inflammatory pathways most consistently associated with T2-low asthma are related to inflammasome activation, including IL-1β and IL-6 signaling,[3] as well as IL-17-mediated neutrophilic inflammation.[3,28] These pathways are likely triggered by environmental factors such as microbes, pollutants, or cigarette smoke.[32] In this context, neutrophils can act as pathogenic effector cells inducing epithelial cell damage and mucus hyperproduction.[3] Given the complexity and heterogeneity of asthma as a disease, it is important to acquire a better understanding of asthma susceptibility and pathophysiology.

3 | GENETIC ASSOCIATIONS WITH ASTHMA

Before the advent of GWAS, decades of work involving classic linkage analysis and candidate-gene approaches have linked numerous genetic loci to asthma.[33,34] Moreover, twin cohort studies have shown that up to 70% of asthma susceptibility originates from genetic factors.[35,36] More recently, GWAS has been used to further explore the genetic basis of asthma. In GWAS, two cohorts of case and control individuals are genotyped on single nucleotide polymorphism (SNP) arrays that include so-called "tag" SNPs that represent haplotype blocks in the genome. These are then compared through statistical methods in order to link genetic variants to specific traits.[37] Since the first asthma GWAS appeared in 2007,[38] the NHGRI-EBI GWAS catalogue now contains 179 published association studies on asthma (both allergic and non-allergic asthma) or asthma-related traits.[6] Table 1 provides an overview of selected "hallmark" GWAS together with their most important findings. Examining the genes in the vicinity of risk SNPs reveals an enrichment for molecules associated with a type-2 immune response, including 2q12 (*IL1RL1*, encoding the IL-33 receptor), 5q22 (*TSLP*), 9p24 (*IL33*), 10p14 (*GATA3*, encoding the master TF for T_H2 differentiation), and 5q31 (*IL4-IL5-IL13*). Many other asthma GWAS genes also have known roles in inflammation, such as *SMAD3*, *BACH2*, *TLR1/6/10*, and *STAT6*. For other associations, biological links with asthma pathophysiology are at first less evident. A prime example is the most widely replicated childhood asthma GWAS locus at 17q21, containing the *ORMDL3* and *GSDMB* genes which encode proteins involved in more general cellular functions, including regulation of apoptosis.[39] Important to note is that most GWAS published to date have used a general asthma definition that makes no distinction between key different endotypes (T2-high versus T2-low). Interestingly, GWAS approaches have revealed substantial overlap between genetic loci associated with asthma and chronic upper airway inflammation (i.e., allergic rhinitis),[40,41] suggesting a shared genetic origin of chronic lower and upper (allergic) airway inflammation and providing support for the united airway disease concept.

Despite these GWAS efforts, a sizeable portion of asthma susceptibility remains unexplained.[59] In one of the largest asthma GWAS published to date, Ferreira et al. showed that common variants as identified by GWAS explain 25.6% of childhood-onset asthma heritability and only 10.6% for adult-onset asthma,[56] in line with findings by Pividori et al.[60] Environmental factors further modulate (genetic) asthma susceptibility. The "hygiene hypothesis" proposes that adoption of a Western diet and a disappearance of frequent exposure to infectious agents leads to dysfunctional immunoregulatory pathways, explaining the dramatic increase in asthma prevalence over the past 50 years in the Western world.[61,62] Recently, this hypothesis has been expanded by the "epithelial barrier hypothesis,"[63] which proposes that urbanization and industrialization leads to an increased presence of epithelial barrier-damaging agents (e.g., from air pollution) that disrupt epithelial barrier function, leading to a host of chronic inflammatory diseases including asthma. Besides acting at the cellular level, environmental factors could also affect asthma susceptibility via gene–environment interactions. For example, the protective effect that growing up on a farm has against asthma was much stronger in children carrying a risk SNP in exon 3 of the *TNFAIP3* gene.[64] Additionally, environmental exposures may affect asthma susceptibility through stable—and possibly heritable—modifications of the genome without altering the DNA sequence—that is, through epigenetics.[65,66]

TABLE 1 Selected key asthma GWAS.

First Author (year)	Discovery sample size	Replication sample size	Reported associations	Main findings
Moffatt MF (2007)[38]	994 EU cases, 1243 EU controls	200 EU cases, 2120 EU controls	1	First asthma GWAS; *ORMDL3/GSDMB* locus as top hit (predominantly associated with childhood asthma)
Hancock DB (2009)[42]	492 MEX case-parent trios	177 MEX case-parent trios	1	One of the first GWAS in Hispanic population; *TLE4*
Gudbjartsson DF (2009)[43]	9392 EU cases, 4458 EU controls	7996 EU cases, 44,890 EU controls	5	First association of *IL1RL1/IL18R*
Kim SH (2009)[44]	84 KOR cases, 263 KOR controls	NA	3	First GWAS on occupational-induced asthma (toluene diisocyanate); Most significant associations in *CTNNA3* gene
Moffatt MF (2010)[45]	10,365 cases, 16,110 controls	NA	10	First meta-GWAS: *HLA-DQ*, *IL33*, *IL2RB*, *SMAD3*
Torgerson DG (2011)[46]	3246 cases, 3385 controls, 1702 case-parent trios, 355 family-based cases and 468 family-based controls (EA, AA, AC, LAT)	2727 EU, 2147 AA and AC, 2299 LAT	7	First time association of *IL1RL1*, *TSLP*, *IL33* in three different ethnic groups novel association in *PYHIN1* in Africans
Ferreira MA (2011)[47]	12,475 EU cases, 19,967 EU controls	3322 EU cases, 22,036 EU controls	4	Replication of Th2 immunity loci in various populations; novel associations in *IL6R*, *LRRC32*
Hirota T (2011)[48]	1532 JAP cases, 3304 JAP controls	5639 JAP cases, 24,608 JAP controls	14	First large Asian asthma GWAS; replication of *MHC* and *TSLP-WDR36*, novel associations on 4q31, 10p14, 12q13
Park BL (2013)[49]	117 KOR cases, 685 KOR controls	142 KOR cases, 996 KOR controls	1	First GWAS on aspirin-induced asthma; *HLA-DPB1* most significant risk factor (shown in functional validation using aspirin challenge with FEV1 decline as readout)
Ferreira MA (2014)[50]	6685 EU cases, 14,091 EU controls	878 EU cases, 2455 EU controls	21	Integration of asthma-related traits (hay fever); previously unreported associations in *ZBTB10* and *CLEC16A*
Bønnelykke K (2014)[51]	1173 EU, 2511 EU controls	395 EU, 2663 EU controls, 6783 cases, 7720 controls	6	Specific study on early childhood asthma and severe exacerbations; strong association in *CDHR3*
Yucesoy B (2015)[52]	74 EU cases, 824 EU controls	NA	180	Only occupational asthma GWAS leading to large number of significant associations
Marenholz I (2015)[53]	1151 EU cases, 10,030 EU controls	1277 EU cases, 7004 EU controls	7	Meta-GWAS relating eczema to asthma; two specific associations: *EFHC1* and *TMTC2-SLC6A15*
Demenais F (2018)[54]	19,954 EU, 107,715 EU, 2149 AF, 6055 AF controls, 1239 JAP cases, 3976 JAP, 606 LAT, 792 LAT controls (TAGC)	NA	69	Large meta-analysis of worldwide asthma GWAS from ethnically diverse populations. First association of *GATA3* locus.
Zhu Z (2018)[41]	25,685 EU allergic diseases cases, 76,768 EU controls	GABRIEL and EAGLE cohorts	77	Large meta-GWAS (>100.000) to find more common variants shared between asthma and allergic diseases
Johansson A (2019)[55]	106,772 EU cases, 239,773 EU controls	83,335 cases, 34,934 controls	383	Very large meta-GWAS (>350.000) identifying 141 loci of which 41 were novel to be associated to asthma, hay fever or eczema
Ferreira MA (2019)[56]	13,962 EU childhood-onset cases and 26,582 EU adult-onset cases. 300,671 EU control cases	CO: 31,759 EU cases vs 214,890 EU controls; AO: 16,297 EU cases vs 217,711 EU controls	179	Study comparing genetic architectures between CO and AO asthma

TABLE 1 (Continued)

First Author (year)	Discovery sample size	Replication sample size	Reported associations	Main findings
Olafsdottir TA (2020)[57]	69,189 EU cases, EU 702,199 controls	NA	88	88 new variants that implicate T cell regulation and airway remodeling; associations include loss-of-function in *TNFRSF8* and gain-of-function *TGFBR1*
Han Y (2020)[58]	64,538 EU cases, 329,321 EU controls + TAGC cohort (Demenais F 2017)	NA	167	Very large meta-GWAS detecting a large number of associations, including an in vivo follow-up study of the CD52 locus

Abbreviations: AA, African-American; AC, African-Caribbean; AF, African; AO, adult-onset; CO, childhood-onset; EU, european; FEV1, forced expiratory volume in the first second; GWAS, genome-wide association study; HIS, Hispanic; JAP, Japanese; KOR, Korean; LAT, Latino; MEX, Mexican; NA, not applicable; TAGC, Trans-National Asthma Genetic Consortium.

4 | ASTHMA: INTEGRATING GENETIC AND EPIGENETIC ASSOCIATIONS

EWAS investigate the relationship between epigenetic modifications and traits and have mostly focused on identifying regions of differential DNA methylation across cohorts, which could in part explain trait susceptibility not captured by GWAS.[67] Importantly, the epigenome—in particular DNA methylation—can be influenced by environmental factors such as air pollution and dietary components.[68] In the context of asthma, the sum of all these external environmental influences (commonly referred to as the "exposome") may thus have a profound effect on asthma susceptibility.[69–71] Important to note is that epigenetic modifications are cell type-specific, and therefore care should be taken when interpreting EWAS results. The first asthma EWAS was published by Stefanowicz et al. in 2012.[72] Since then, the National Genomics Data Center EWAS Atlas contains 12 published asthma EWAS[73] (summarized in Table 2).

Differentially methylated cytosine-phosphate-guanine (CpG) nucleotides (DMCs) were identified in non-coding regions near genes previously associated with asthma by GWAS, including *SMAD3*, *IL5*, and *ORMDL3*, revealing significant colocalization with GWAS SNPs.[76,77] Integration of GWAS and EWAS findings has the potential to provide new insights into asthma susceptibility and disease mechanisms. To this end, we integrated asthma GWAS and EWAS data and found a moderate proportion of GWAS- and EWAS-associated genes to overlap (n = 609), which were mostly involved in T cell differentiation and activation, cell migration and inflammation (Figure 2A and Figure 2B). Among these overlapping genes were most of the canonical asthma-associated genes such as *GATA3*, *ORMDL3*, and *IL5*.[33] Genes specifically reported by GWAS were also strongly enriched for immune and inflammatory processes (including T cell responses), whereas genes only detected in EWAS were associated with cell–cell adhesion, cell junction organization, and growth factor signaling (Figure 2B). These differences are likely (in part) caused by the cell type-specific nature of DNA methylation (in contrast to cell type-invariant genetic profiles), since most EWAS were performed on epithelial cells or whole blood samples. Both GWAS and EWAS variants show a predominant localization to distal non-coding regions far from transcription start sites (Figure 2C and Figure 2D), where they are expected to influence gene regulation. Interestingly, CpG islands that are located in these distal regions can exert substantial enhancer activity.[78]

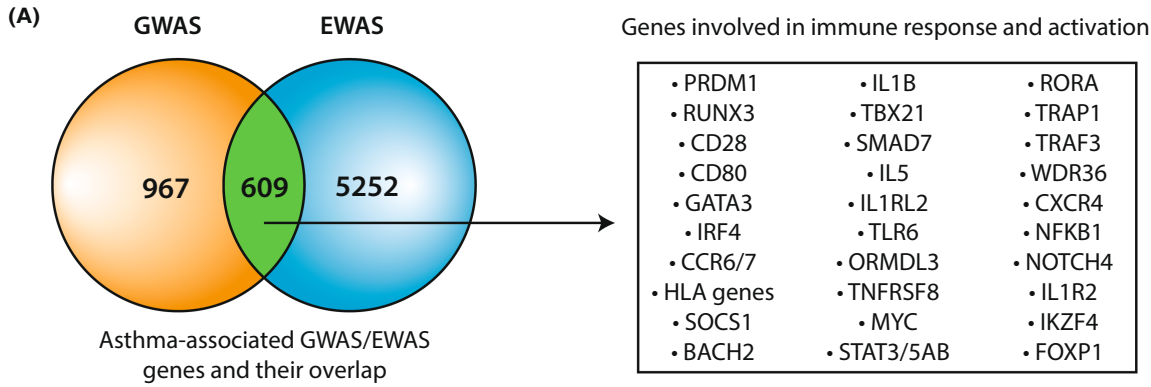
5 | LINKING VARIANTS TO CHANGES IN GENE EXPRESSION: QUANTITATIVE TRAIT LOCUS ANALYSIS

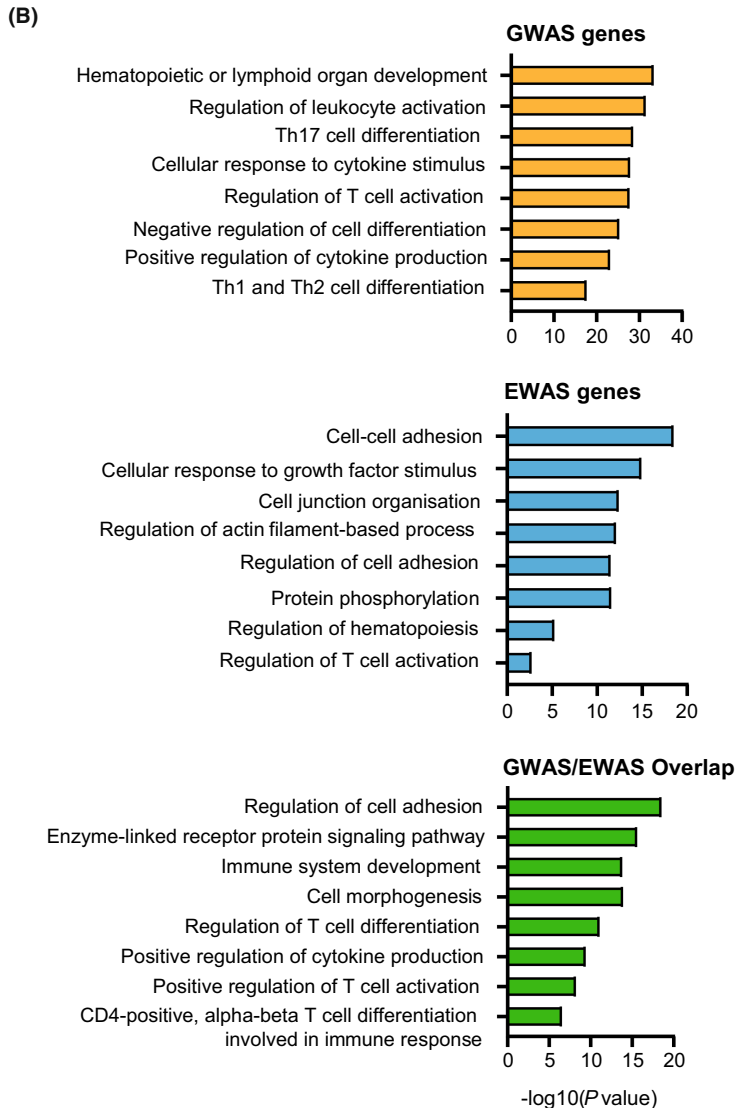
The localization of asthma-associated variants in non-coding regions precludes the direct determination of causal variant–gene relationships. Hence, an important next step is to link (epi)genetic variants to differences in gene expression. A classic statistical approach to uncover the relationship between a phenotypic trait and genotype is by

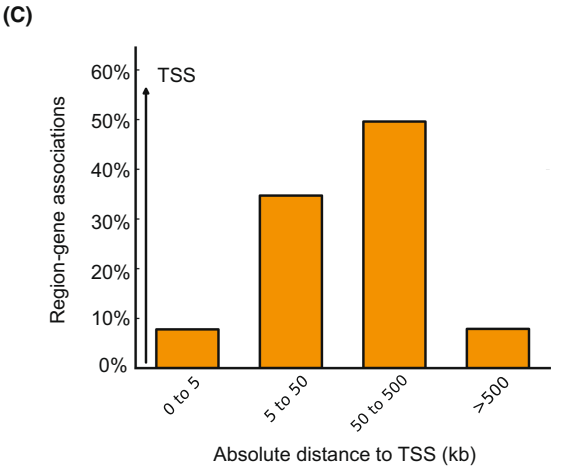
TABLE 2 Selected asthma EWAS

First Author (year)	Sample size (cell type analyzed)	No. of CpGs found	Methylation platform	Feature
Stefanowicz (2012)[72]	5 cases and 20 controls (AECs and PBMCs)	6 CpGs in asthmatics compared to healthy controls in PBMCs. 8 CpGs in asthmatic compared to atopics (AECs)	Illumina GoldenGate Methylation Cancer Panel I	First asthma EWAS; *STAT5A* and *CRIP1* loci implicated
Nicodemus-Johnson (2016)[13]	74 asthmatics and 41 healthy controls (AECs)	40,892 CpGs	Illumina Infinium HumanMethylation 450k BeadChip	Large asthma EWAS; regulatory region for *ORMDL3* found
Xu (2018)[62]	392 cases and 1156 controls (replicated with 247 cases and 2949 controls) (Whole blood)	14 CpGs	Illumina Infinium HumanMethylation 450k BeadChip	Large implications for Th2 immunity; associations with CD4/CD8 T cells and eosinophils
Reese (2019)[14]	668 newborns (8 case–control cohorts); cross-sectional 631 cases (9 case–control cohorts) (whole blood/NEB)	188 CpGs	Illumina Infinium HumanMethylation 450k BeadChip	Largest meta-EWAS to date; associations with *IL5RA* and *KCNH2*
Qi (2020)[74]	72 whole blood and 97 nasal brush persistent asthma cases and 636 controls, replicated in Dutch Lifelines population-based cohort and French EGEA case–control cohort	7 CpGs for clincial remission and 129 CpGs for complete remission in whole blood	Illumina Infinium HumanMethylation 450k BeadChip	EWAS replicated in different ethnicities, linking DMRs associated to asthma with animal dander exposure
Jiang (2021)[75]	Meta-analysis of PR-GOAL and EVA-PR cohorts (601 Puerto Rican case–control subjects) and GALA II cohort (5147 Latino case–control subjects)	195 CpGs	Illumina Infinium HumanMethylation 450k BeadChip	First meta-EWAS in Latino children; Top hits include *CAMK1D* and *TIGIT*; associations with TGF-β pathway

Abbreviations: AA, african-american; AEC, airway epithelial CellAsRh; CpG, cytosine-phosphate-guanine; DMR, differentially methylated region; NEB, nasal epithelial brush; PBMC, peripheral blood mononuclear cells; PR, puerto-rican.







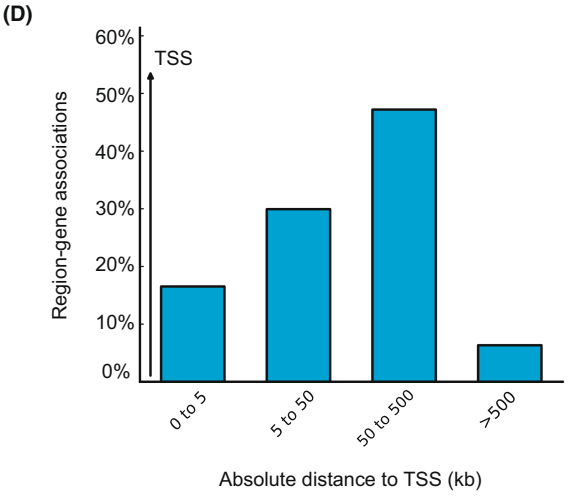


FIGURE 2 Comparison of genetic and epigenetic variants associated with asthma. (A) Venn diagram integrating reported genes from asthma GWAS and EWAS, highlighting overlapping genes important in immune development and activation. The NHGRI-EBI GWAS catalogue currently counts 3129 genetic variants associated with asthma and childhood traits.[6] These 3129 variants annotate to 1576 genes bases on nearest gene using Genomic Regions Enrichment of Annotations Tool (GREAT).[79] The NGDC EWAS Atlas contains 13,677 CpGs associated to asthma,[73] which are annotated to 5861 genes (using the *getMappedEntrezIDs* command of the *missMethyl* R package[80]). (B) Pathway enrichment analysis of genes detected only by GWAS, EWAS or in both GWAS and EWAS ("overlap") using Metascape.[81] (C) Absolute distance (in kilobases) of asthma-associated genetic variants to the transcription start site (TSS) of the nearest gene, performed using GREAT. (D) Absolute distance (in kilobases) of asthma-associated DMCs to the TSS of the nearest gene, performed using GREAT.

performing a quantitative trait locus (QTL) analysis.[82] One popular variant of QTL mapping is to focus on the subset of GWAS SNPs that correlates with altered gene expression levels, which are referred to as expression quantitative trait loci or eQTLs.[83,84] eQTLs are identified by measuring gene expression in samples from a cohort of genotyped individuals. Since gene expression programs are highly cell type- and cell state-specific,[85,86] samples selected for gene expression measurements should be relevant for the trait or disease under investigation. Efforts to identify eQTLs for specific diseases have been greatly aided by the generation of large eQTL databases for specific non-diseased tissue samples (e.g., GTEx[87]). In keeping with the observation that GWAS SNPs often localize far from genes, cell type-specific eQTLs also localize to distal GREs. Similarly, DNA methylation QTL (meQTL) analysis has been conducted in order to link EWAS DMCs to altered gene expression.[88] Hereby, recent findings indicate that eQTLs and meQTLs often occur at the same genomic location.[89]

In the context of asthma, various (m)eQTL analyses have been conducted.[13,90–95] For example, Ketelaar et al. performed an eQTL analysis of the *IL33* locus and found two SNPs, both associated with eosinophilic asthma, which were eQTLs for *IL33* expression in bronchial brushes and cultured bronchial epithelial cells (BECs). Both risk alleles were associated with increased levels of *IL33* mRNA.[96] Li et al. integrated GWAS and gene expression data from human BECs (n = 107) and bronchial alveolar lavage samples (n = 94) for eQTL analysis. They identified several GWAS SNPs linked to altered expression of various asthma-related genes, including *TSLP*, *GSDMB*, *IL33*, and *HLA-DQB1*.[91] Furthermore, a recent study integrated eQTL and meQTL data from upper airway epithelial cells with GWAS SNPs to pinpoint SNPs with potential impact on gene regulatory mechanisms.[97] These examples illustrate how (m)eQTL analyses can prioritize GWAS/EWAS findings as potential causal hits that affect the expression of a disease-relevant gene. Beyond eQTLs and meQTLs, other *cis* or *trans*-acting mechanisms such as DNase hypersensitivity (dsQTLs), TF binding (bQTLs) and histone modifications (hQTLs) are also used in translating GWAS findings to biological mechanisms for various diseases - including asthma.[98–102]

Transcriptome-wide association studies (TWAS) have emerged as yet another approach for linking GWAS variants to altered gene expression. In a TWAS, gene expression reference datasets (e.g., the GTEx eQTL database[87]) are integrated with GWAS findings to identify gene-trait associations. Predictive models of expression variation are generated for each gene using allele counts of genetic variants near the gene, allowing one to predict gene expression for individuals in a GWAS cohort and link expression levels to specific traits.[103,104] Various TWASs have been performed for asthma.[95,105–107] For example, a TWAS done in nasal respiratory epithelium from individuals with childhood asthma identified differentially expressed genes enriched for pathways involved in the induction of T_H1/T_H2 differentiation, antigen presentation, and metabolism.[108] TWAS was also able to yield new asthma-gene associations, as whole blood eQTLs were used to identify 4 reproducibly affected novel asthma genes involved in nucleotide synthesis and nucleotide-dependent cell activation.[25] Although TWAS does not inform on the exact causal genetic variants, when combined with additional fine-mapping strategies, TWAS is a powerful method for elucidating the mechanisms underlying GWAS findings.[109] An online collection of TWAS data can be found in the TWAS hub (http://twas-hub.org).[110]

6 | EPIGENOMICS TO PINPOINT PUTATIVE CAUSAL VARIANTS

The non-coding localization of trait-associated variants is not the only challenge that complicates the interpretation of GWAS findings. Another main hurdle is linkage disequilibrium (LD), which refers to the linked heritability of neighboring genetic variants in the genome as they co-segregate through meiosis. GWAS in fact make use of this: by focusing on a limited set of variants that represent regions of high LD ("tag SNPs"), modern GWAS can assay genome-wide for genotype–phenotype associations without having to analyze every individual SNP. However, many SNPs will reside in high LD with tag SNPs, thus often obscuring the identification of true causal variants. Hence, GWAS associations generally do not implicate single variants but rather identify regions of high LD linked to the trait under investigation.

Making biological sense of non-coding genetic variants or CpG methylation changes is greatly facilitated by integrating epigenomics data.[99] Functional effects of (epi)genetic variants are - similar to eQTLs—highly cell type-specific, since transcriptional regulation is orchestrated by cell type-specific GREs such as enhancers.[111] In addition, the activation status of certain cell types, in particular immune cells, is reflected in highly dynamic epigenomes and transcriptomes.[85,112,113] Epigenomics assays such as Assay for Transposase-Accessible Chromatin (ATAC)-Seq[114] and chromatin immunoprecipitation followed by sequencing (ChIP-seq) can readily identify GREs as well as provide information on their activation status.[115] For instance, GREs poised for activation comprise of open chromatin flanked by nucleosomes with histone 3 lysine 4 monomethylation (H3K4me1) or H3K4me2, whereas fully active GREs are marked by histone 3 lysine 27 acetylation (H3K27Ac).[11] It is within these cell type- and state-specific GREs that (QTL) SNPs can exert their effects. For example, genetic variants can disrupt DNA binding motifs recognized by TFs, potentially resulting in aberrant GRE activity and misregulated gene expression.[116]

Numerous studies have mapped GWAS SNPs to cell type-specific GREs, supporting the concept of disease-associated "regulatory SNPs" underlying human traits and disease.[9,89,91,93,117] For asthma, epigenomics data have been used to link enhancer regions in $CD4^+$ T cells, ILC2s, and BECs to asthma susceptibility.[58,118–121] For example, Seumois et al. used H3K4Me2 ChIP-Seq of circulating Th2 cells to show that GWAS SNPs are enriched in Th2-specific GREs, and that some of these GREs exhibited differential H3K4Me2 levels in cells from asthma patients compared with healthy controls.[118] Notably,

many GWAS variants localize to stimulation-responsive GREs, highlighting the critical importance of including epigenome information from activated (immune) cells.[112,113,122] Indeed, we showed that asthma-associated genetic variation is concentrated in H3K4Me2+ putative GREs in ILC2s activated by epithelial alarmins IL-25 and IL-33, revealing both shared and unique SNP-GRE colocalization with those observed in Th2 cells.[119] Of note, altered histone modifications and regulatory protein binding have also been linked to asthma pathophysiology outside the context of (epi)genetic risk variants.[123] For example, studies in epithelial cells from asthmatics and healthy controls revealed widespread changes in histone acetylation, indicating changes in gene regulatory mechanisms in the airways of asthma patients.[124]

Enhancers are often located at large genomic distances from their target genes, regulating gene expression at long range through spatial interaction (or "chromatin looping") with promoter regions.[125–127] Hence, enhancers do not necessarily regulate the expression of the nearest gene, which poses a problem for linking SNP-GRE combinations to candidate genes. Together with QTL data, chromosome conformation capture (3C) methods that measure spatial proximity between genomic regions can be highly informative.[128] Combined with functional assays to directly test whether a SNP affects GRE activity, detailed analysis of the epigenome at disease-associated loci provides a first step at identifying the mechanistic basis of a (epi) genetic association.

7 | A GENERIC WORKFLOW FOR FOLLOW-UP STUDIES OF TRAIT-ASSOCIATED (EPI)GENETICS VARIANTS

Figure 3 provides a schematic overview of a generic approach to translate GWAS and EWAS findings to biological insights, highlighting the power of epigenomics for prioritizing candidate SNPs, GREs and cell types. An initial effort involves retrieving GWAS/EWAS variants from public databases and literature, followed by—for GWAS data—the imputation of SNPs based on LD structure. To this end, we highly recommend using the FUMA web application,[129] which provides an intuitive way to identify independent significant SNPs and directly includes SNPs in high LD.

The resulting list of variants can be intersected with publicly available QTL and epigenomics datasets to obtain a refined list of candidate variants (Figure 3). Since transcriptomic and epigenomic information is cell type- and state-specific, it is critical to use data obtained from trait-relevant cell types (e.g., Th2 cells or lung epithelial cells in the case of asthma). Fortunately, easily accessible collections of eQTL or epigenomic information—including 3C-type data—from numerous tissues and cell types are currently provided by large-scale community efforts such as the GTEx, ENCODE, BLUEPRINT and Epigenome Roadmap consortia.[87,130–134] Subsequently, variants sets can be further refined using computational scoring matrices such as the Combined Annotation Dependent Depletion (CADD) tool[135] or RegulomeDB tool.[136] Of note, FUMA offers a simple means to intersect eQTL and epigenomics data from various sources to comprehensively annotate a set of GWAS variants,[129] as we have recently exploited in an effort to functionally annotate genetic variants associated with severe COVID-19.[137]

After the initial list of tag and LD SNPs has been reduced to a smaller list of putative causal SNPs based on QTL and regulatory SNP annotations (Figure 3), several experimental approaches exist to further strengthen the functional relationships between candidate variants, GREs and target genes. To test whether a GRE has enhancer or promoter activity, in vitro or in vivo reporter assays can be used. In these assays, the putative GRE is coupled to a reporter gene (e.g., firefly luciferase) in a DNA construct (e.g., a plasmid or bacterial artificial chromosome). Measuring reporter gene expression provides a transcriptional read-out of GRE activity in a given cell type. While traditionally laborious and low throughput in nature, the recent development of massively parallel reporter assays (MPRAs) now offers a high throughput means to test thousands of putative GREs in a single experiment.[138] When applied to GWAS SNPs, reporter assays (including MPRAs) offer a powerful approach that can simultaneously determine the regulatory activity of SNP-enriched GREs and the impact of the variant by testing both risk and protective alleles.[83,139–141] In addition to plasmid-based reporter approaches, CRISPR-Cas9-based approaches can also be used to determine the gene regulatory role of non-coding regions in the genome in a more native chromatin context, as opposed to the more artificial plasmid approach.[142] For example, CRISPR-Cas9 can be used for base editing purposes in order to test the effect of a SNP on gene regulation, as has been done in the context of asthma.[143] Complementary to these approaches, TF motif analyses can assess whether specific motifs may be enhanced or disrupted by the putative causal SNP. Conveniently, several publicly available analysis platforms have been created to computationally intersect GWAS SNPs with type-specific GREs and/or TF-binding motifs, including HaploReg,[144] GWAS4D,[145] the FUMA platform[129] and RegulomeDB.[136]

Various studies have (in part) followed the Figure 3 workflow. For example, we previously investigated intergenic 6q23 variants linked to clinically relevant red blood cell parameters, showing that these SNPs impaired TF binding, weakened enhancer–promoter interactions and lower expression of *MYB*—encoding a TF essential for erythropoiesis.[146] Using similar approaches, Miller et al. identified SNPs associated with coronary artery disease that disrupted TF binding and transcriptional regulation of genes important for smooth muscle cell biology.[147] Additionally, obesity-associated variants within the *FTO* locus were shown to disrupt a long-range enhancer of *IRX3*, a gene critical for controlling body mass in mice.[148] Importantly, this GWAS follow-up strategy has already resulted in the development of new therapies. Bauer et al. reported that common genetic variation in an intron of *BCL11A* associated with elevated fetal hemoglobin levels disrupts TF binding to an erythroid-specific enhancer of *BCL11A*, effectively

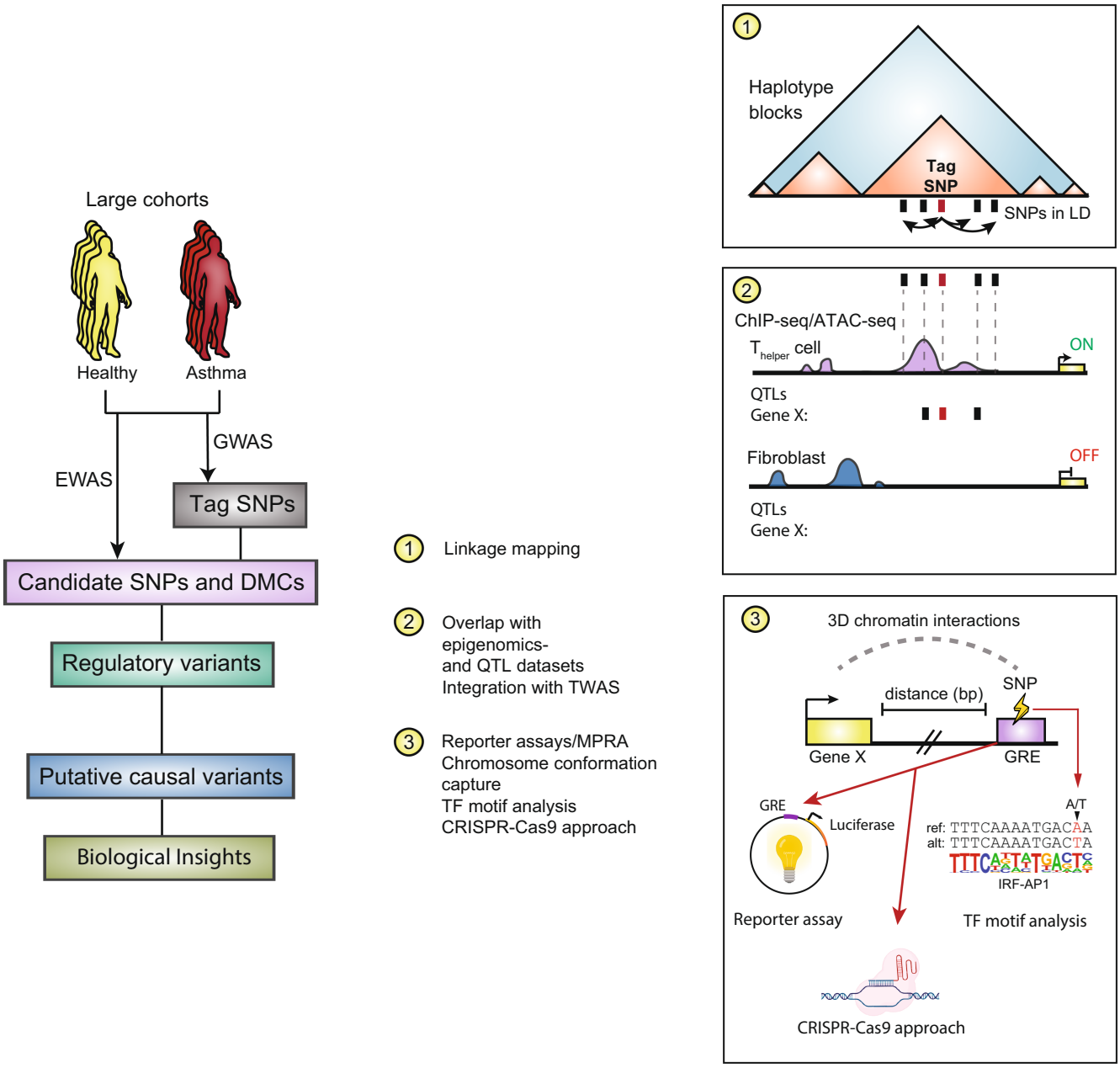


FIGURE 3 Schematic overview of a generic workflow to link non-coding GWAS/EWAS associations to gene regulatory mechanisms in specific cell types. Tag SNPs and LD SNPs from GWAS integrated with DMCs from EWAS studies provide a list of candidate SNPs and DMCs. Variants can subsequently be prioritized by filtering SNPs with QTL analyses and/or to regulatory regions in the genome. The latter is illustrated by a schematic example of a ChIP-Seq and ATAC-seq-based approach to prioritize SNPs that reside in putative enhancer regions of T_{helper} cells or fibroblasts, which are marked by H3K27Ac. This significantly reduces the number of candidate causal SNPs and assigns (a lack of) cell-type specificity to the remaining variants. The resulting list of putative causal variants can then be validated in in vitro or in vivo based experiments such as reporter assays combined with chromosome conformation capture assays, CRISPR-Cas9-based approaches and transcription factor binding motif analyses, in order to gain biological insight into the regulatory function of the tested variants. Abbreviations: DMC, differentially methylated CpG; GRE, gene regulatory element; MPRA, massively parallel reporter assay; QTL, quantitative trait locus; SNP, single nucleotide polymorphism; TF, transcription factor; TWAS, transcriptome-wide association Study.

reducing BCL11A levels and as a direct consequence increasing fetal hemoglobin levels.[149] Gene therapy targeting this *BCL11A* enhancer has now been successfully used to treat patients with adult hemoglobin defects (e.g., sickle cell disease).[150] Thus, a combination of epigenetics, QTL analyses and experimental validation can translate GWAS findings into disease-relevant biological insights.

8 | FOLLOW-UP STUDIES OF MAJOR ASTHMA-ASSOCIATED GENETIC VARIANTS

Functional studies into the biological mechanisms underlying genetic risk loci for asthma remain relatively scarce. To date, only a handful of asthma-associated loci have been subjected to more

in-depth follow-up experiments aimed at establishing causal variant-gene relationships with potential relevance for asthma pathophysiology. A major focus of these studies has been the 17q12-21 locus (reviewed in[151]), which was first identified by Moffatt et al. in 2007[38] and consistently detected as one of the strongest associations in subsequent GWASs, particularly in childhood asthma. eQTL analyses revealed that 17q12-21 variants are strongly associated with increased *GSDMB* and particularly *ORMDL3* expression in blood cells and lung tissue, with no or weaker associations detected with other nearby genes.[151] Although not always consistent, gene expression studies point towards increased *ORMDL3*–and usually not *GSDMB*—expression in PBMCs or bronchial epithelial cells from asthma patients.[151–153] Interestingly, SNPs located in other (homologous) members of the ORMDL family were also linked to asthma.[154] Rhinovirus exposure in adults induced *ORDML3* and *GSDMB* expression in PBMCs, further linking the 17q12-21 locus to respiratory virus infections associated with increased risk to develop asthma.[155] Follow-up studies using human lymphoblastoid cell lines[156] or primary immune cells[121] showed that 17q21-31 variants localize to GREs and affect local chromatin accessibility as well as TF binding–particularly in primary T cells.[121] In human CD4+ T cells, two candidate causal SNPs (rs12936231 and rs4065275) altered binding of the CTCF insulator protein, rewiring interactions between enhancers and *ORDML3* that result in increased expression levels. Higher levels of ORDML3 repressed the production of IL-2, a critical regulator of Th cell function, providing a plausible link between 17p21-31 variants and asthma susceptibility.[121] In addition, EWAS identified a DMC (cg05616858) in a putative GRE associated with asthma and *ORDML3* expression.[13] However, in vivo models of allergic asthma using transgenic mice in which ORDML3 levels were altered have generated conflicting results,[157,158] and the relationship between ORDML3 levels, respiratory viral infections and asthma development was not addressed in these studies. Hence, more research is required to unravel the possible causal relationship between 17p21-31 variants, ORDML3, and asthma pathogenesis.

Both the *IL33* and *IL1RL1* (encoding the IL-33 receptor) loci have also been investigated in more detail. GWAS variants in the *IL33* locus are associated with increased *IL33* expression in human BECs and increased eosinophil levels, with several SNPs residing in putative GREs where they may modify TF binding sites.[96] Aneas et al. used fine-mapping and functional genomic analyses in mice, zebrafish and human cell lines to pinpoint rs1888909 as a putative causal variant.[159] Rs1888909 creates an OCT-1 TF binding site postulated to interfere with an insulator GRE that controls *IL33* expression, in agreement with increased epithelial *IL33* mRNA expression and elevated IL-33 plasma protein levels in carriers of the rs1888909 risk alleles.[159] Increased IL-33 production in airway epithelial cells could aggravate type-2 inflammation, explaining the genetic association with increased risk of asthma development. Studies of the *IL1RL1* locus in primary human cells have painted a more complex picture, pinpointing missense coding but also non-coding eQTL SNPs associated with the expression of both *IL1RL1* isoforms, that is, membrane-bound and soluble IL1RL1 (the latter acting as a decoy inhibitory receptor).[160–166] Variants associated with either increased or decreased *IL1RL1* isoform expression were linked to asthma susceptibility. Moreover, eQTL effects were highly cell type-specific with substantial differences across airway epithelial cells, endothelial cells, and T cells. How non-coding variants affect *IL1RL1* regulation remains unclear, although the *IL1RL1* promoter regions appear particularly enriched for GWAS SNPs, as was shown for activated human ILC2s[119] and for human BECs from asthma patients.[120]

Variants near *TNFAIP3* (6q23) have been associated with multiple inflammatory disorders, including asthma.[167] *TNFAIP3* encodes A20, a critical attenuator of pro-inflammatory NF-κB signaling.[168] Most follow-up efforts have been conducted in the context of autoimmunity. The rs6927172 variant emerged from multiple studies, localizing to an enhancer element active in human myeloid cells and T cells that engages in long-range interactions with the *TNFAIP3* promoter.[112,141,167,169,170] Mechanistically, rs6927172 disrupts an NF-κB binding site required for enhancer activity and *TNFAIP3* transcription—explaining the reduced *TNFAIP3* levels associated with the risk allele.[112,141,169] Indeed, Schuijs et al. provided compelling evidence that the immune-suppressive action of A20 is critical for increasing the threshold for developing allergic asthma in mice, which was further supported by reduced *TNFAIP3* expression in lung epithelial cultures of (severe) asthmatics.[64] Notably, an exonic SNP in *TNFAIP3* (rs2230926) was associated with allergy and asthma risk in children,[64] suggesting multiple mechanisms through which common genetic variation could affect A20 protein function.

Other asthma-associated loci from GWAS studies have received less attention. The 5q31 GWAS signal spanning the Th2 cytokine locus was shown to harbor a SNP (rs2240032) that is located in the Th2 locus control region (LCR), a cluster of enhancer elements that have been extensively characterized in mouse models as a critical driver of *IL4*, *IL5*, and *IL13* transcription in Th2 cells.[171,172] Rs2240032 was suggested to influence *IL4* expression and *IL13* promoter methylation in cord blood cells, which may be relevant for asthma pathogenesis given the central role of these cytokines in the development of allergic and T2-high asthma.[173] 5q31 variants may also affect type-2 cytokine production by other cell types, including human ILC2s.[119] Han et al. recently used an eQTL-informed approach to prioritize candidate causal variants near immune-associated genes, arriving at the *CD52* gene (1p36.11) that encodes a membrane protein present on various immune cells with a known role in T-cell activation.[58] Anti-CD52 antibody treatment in a mouse model of allergic asthma ameliorated inflammatory symptoms in vivo, providing evidence that *CD52* is indeed a causal gene for asthma risk.[58] Finally, Olafsdottir et al. reported a prioritized missense variant (rs2230624) in *TNFRSF8* and a 3′ UTR variant in *TGFBR1* (rs41283642) associated with decreased asthma risk.[57] Rs2230624 reduced the protein surface expression and shedding in a human cell line model system of TNFRSF8 (also known as CD30), a co-stimulatory molecule expressed on T cells that is important for the induction of allergic airway inflammation in mice.[174] Rs41283642 alters the recognition site of the miR-142-3p microRNA,[57] which mediates regulation of TGF-β signaling and may thus contribute to asthma pathogenesis.[175]

Altogether, several excellent GWAS follow-up studies have started to elucidate the impact of candidate causal genetic variants on the regulation of genes (potentially) relevant for asthma pathophysiology. However, the vast major of associated loci remain unexplored and current efforts have predominantly focused on protein-coding genes, despite the clear relevance of non-coding RNA species such as microRNAs and long non-coding RNAs in asthma pathogenesis[176,177]

9 | CONCLUSIONS AND FUTURE DIRECTIONS

Asthma GWAS have identified 3129 SNP-trait associations linked to >140 loci, and EWAS has collectively identified thousands of disease-associated epigenetic variants. Increasing the size of GWAS/EWAS efforts will undoubtedly uncover additional asthma-associated variants and genes,[178] although the extensive clinical heterogeneity of asthma as well as the underrepresentation of populations of non-European descent represent significant challenges that future studies need to address. Performing GWAS/EWAS on homogeneous populations of more therapy-resistant T2-low, adult onset, obesity-associated asthma or asthma with specific comorbidities could yield endotype-specific variants and offer novel biological insights.[178] Indeed, well-powered GWAS have for example identified genetic variants specifically linked to childhood or adult-onset asthma[56,60,179] and a smaller scale study identified (m)eQTLs specifically associated with obesity-associated asthma in children. In addition, well-powered and ethnically diverse studies also have the potential to improve the power of common variants to identify individuals at risk of developing asthma via polygenic risk scores, which can be used in the clinic for patient stratification and precision medicine approaches.[179,180] Through such approaches, GWAS/EWAS findings may also prove valuable as biomarkers in a clinical context, for example, by supporting patient phenotyping or for predicting risk of developing exacerbations and therapy response.[33,181,182] Given that cost of (epi)genotyping assays have been rapidly decreasing, we envision that well-curated sets of (epi)genetic variants could in the future be employed in various clinical settings. Regarding EWAS findings, it should be noted that replication of these associations has remained challenging, likely due to relatively small cohort sizes and differences in the investigated cell types or tissues.[76]

Perhaps, the most significant challenge that we now face is how to capitalize on the wealth of available asthma GWAS/EWAS data. Given that human genetics evidence supports two-third of the 2021 FDA-approved drugs[183] and that drug targets with genetic support are twice as likely to get FDA approval,[184] we believe it is important for the field to focus on the functional dissection of asthma-associated variants. Indeed, current asthma treatment modalities include biologicals that act on proteins that have been extensively associated to asthma in GWAS and EWAS data, including IL-5, IgE, and TSLP.[185–188] However, very few associations have been thoroughly investigated to determine which variant(s) are causal, what molecular mechanisms underlie the impact of the genetic variants, and which biological pathways relevant for the risk of developing asthma are affected. Furthermore, in order to fully understand the mechanistic basis of asthma it is crucial to validate findings from association studies in more sophisticated models like animal or primary cell-based models, rather than remaining at the association level. It will be important in this endeavor to not only focus on genes and pathways that are well-known to be relevant for asthma pathogenesis. Rather, loci should be explored with less clear links to the disease, as these may provide novel entry points for biomarker or therapy development.[57,58] With an ever-increasing capacity for "omics" data generation and analysis, we propose to take full advantage of publicly available epigenomics and QTL resources to prioritize non-coding variants for functional follow-up studies to arrive at actionable biological processes relevant for asthma pathophysiology.

AUTHOR CONTRIBUTIONS

B.S., R.W.H., and R.S. conceptualized and wrote the review.

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CONFLICT OF INTEREST STATEMENT

All authors have read and approved the manuscript. There are no conflicts of interest.

ORCID

Bernard S. Stikker https://orcid.org/0000-0002-3040-4562
Ralph Stadhouders https://orcid.org/0000-0002-1060-5607

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