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Review Article

Omics approaches in asthma research: Challenges and opportunities

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ABSTRACT

Asthma, a chronic respiratory disease with a global prevalence of approximately 300 million individuals, presents a significant societal and economic burden. This multifaceted syndrome exhibits diverse clinical phenotypes and pathogenic endotypes influenced by various factors. The advent of omics technologies has revolutionized asthma research by delving into the molecular foundation of the disease to unravel its underlying mechanisms. Omics technologies are employed to systematically screen for potential biomarkers, encompassing genes, transcripts, methylation sites, proteins, and even the microbiome components. This review provides an insightful overview of omics applications in asthma research, with a special emphasis on genetics, transcriptomics, epigenomics, and the microbiome. We explore the cutting-edge methods, discoveries, challenges, and potential future directions in the realm of asthma omics research. By integrating multi-omics and non-omics data through advanced statistical techniques, we aspire to advance precision medicine in asthma, guiding diagnosis, risk assessment, and personalized treatment strategies for this heterogeneous condition.

Introduction

Asthma, a chronic respiratory disease characterized by airway inflammation and hyperresponsiveness, encompasses diverse clinical symptoms including wheezing, coughing, and dyspnea.[1,2] Over the past few decades, there has been a substantial global increase in asthma prevalence across both pediatric and adult populations.[3,4] Current research indicates that approximately 300 million individuals worldwide are affected by asthma.[5] The overall prevalence of asthma in children, adolescents, and adults was estimated to be 9.1%, 11.0%, and 6.6%, respectively.[2] Asthma poses a significant burden on society and the economy due to the costs associated with medical care and the impact on work or school attendance.[6–10] Furthermore, severe acute exacerbations of asthma can lead to hospitalization and even mortality.[11–14] Thus, there is an urgent need for effective personalized clinical management strategies and preventive measures to mitigate the impact of asthma, particularly in vulnerable populations such as children.

As a heterogeneous syndrome, asthma has diverse clinical phenotypes and pathogenic endotypes which are influenced by multiple factors such as age, gender, and complex interactions of genetic and environmental components.[15,16] The traditional classification of asthma into allergic or non-allergic categories is deemed overly simplistic for efficacious clinical management.[17] Allergic asthma is characterized by a T helper 2 (Th2) cell-mediated response in which allergen-specific Th2 cells generate type 2 cytokines (e.g., interleukin [IL]-4, IL-5, and IL-13), resulting in significant eosinophil infiltration in the airway wall and elevated immunoglobulin E (IgE) synthesis.[18] T cell inflammatory pathways are considered to play a vital role in the pathogenesis of asthma. The development of novel treatments targeting type 2 inflammation pathways highlights the need for clinical management strategies that consider underlying asthma endotypes driving the disease in individuals.[19,20] Previous studies have identified two main endotypes of asthma based on the degree of type 2 inflammation, Th2-high (T2-high) and Th2-low (T2-low/Non-T2), each exhibiting distinct responses to therapeutic interventions.[21–23] Omics research offers in-depth molecular assessments of asthma patients, identifying biomarkers associated with endotypes, uncovering novel mechanisms involving genes, proteins, metabolites, or microbiota in asthma progression, and facilitating the development of precise treatment.[24] Fig. 1 illustrates how multi-omics and non-omics data are integrated through statistical methods to boost precision medicine in asthma, guiding diagnosis, risk assessment, and tailored treatments.

Omics techniques have significantly enhanced our understanding of the molecular mechanisms underlying asthma. For example, genome-wide association studies (GWASs) have identified numerous asthma susceptibility loci relevant to specific populations.[25–28] Epigenome-wide association studies (EWASs) have revealed distinct

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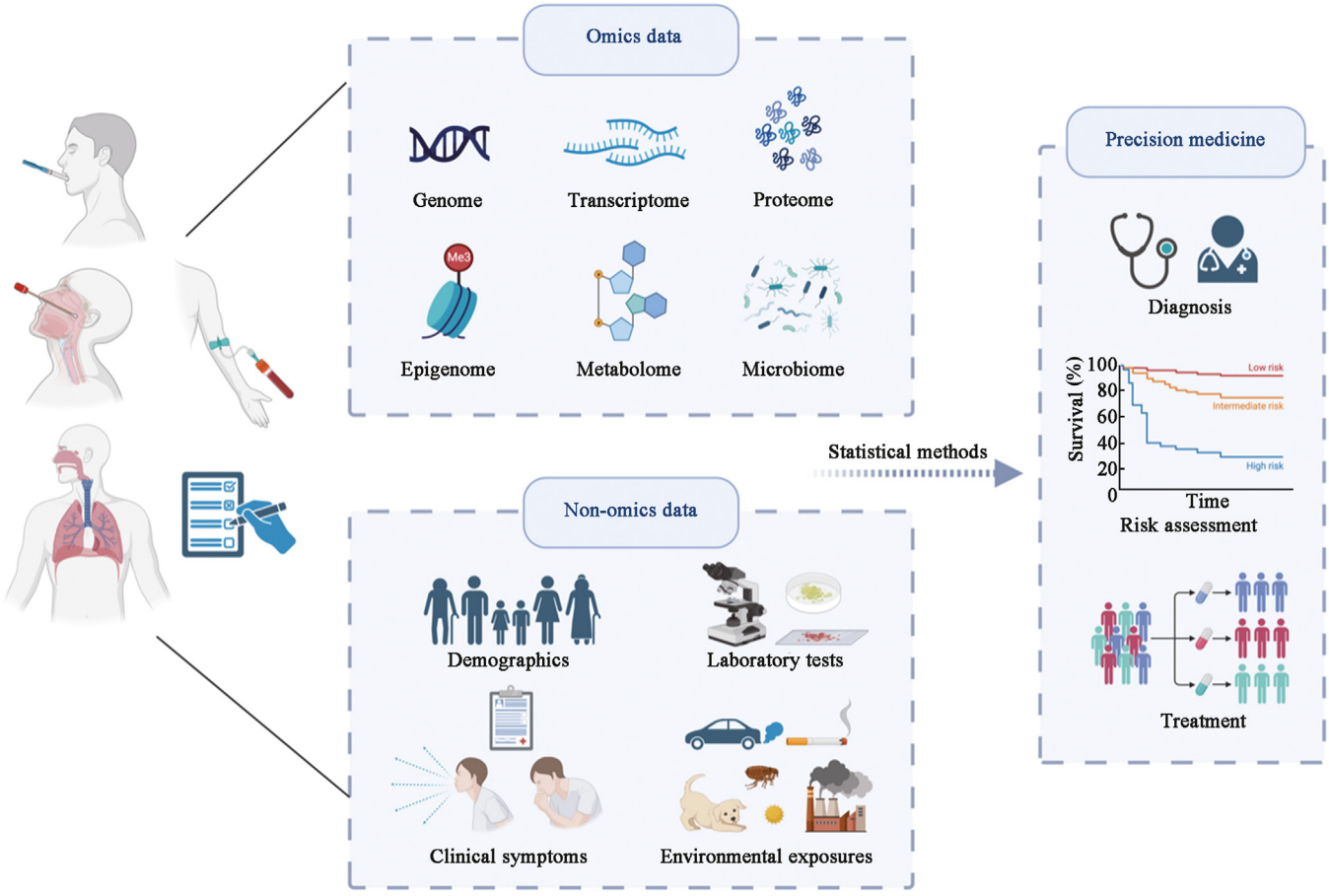

Fig. 1. Overview of multi-omics in asthma, which aids in assessing the risk of asthma, defining endotypes, and predicting responses to specific treatments. Figure was created with Biorender.com.

deoxyribonucleic acid (DNA) methylation patterns associated with asthma.[29–32] During the course of asthma, levels of proteins associated with inflammation, cell apoptosis, and proliferation also change. Through analyzing protein expression, proteomics offers a deeper understanding of the pathophysiological mechanisms behind bronchial asthma.[33] The U-BIOPRED project has employed transcriptomics, proteomics, lipidomics, metabolomics, and clinical phenotypes in asthma patients to identify multiple asthma endotypes and genes that are associated with inflammatory pathways.[34–36] Omics approaches have demonstrated the capacity to provide unprecedented insights into the identification of asthma endotypes and associated biomarkers, which is especially important for pediatric asthma.

In this article, we aim to provide an overview of omics approaches utilized in current asthma research, primarily focusing on genetics, transcriptomics, epigenomics, microbiome, as well as their integration. We summarize the state-of-the-art advances in omics for asthma research, discussing cutting-edge research methods, discoveries, challenges, and potential future directions.

Genetics

The asthma genetics studies mainly focus on the role of DNA in disease onset, progression, drug responses, and prognosis. Genetic research samples could be obtained from any nucleated tissue, typically using blood or saliva, which are convenient to obtain.[24] Individual genotyping is typically performed using microarrays or next-generation sequencing (NGS) methods, such as whole-genome sequencing (WGS) and whole-exome sequencing (WES). Due to the high cost of NGS, microarray-based genotyping remains the most commonly used method.[37]

GWASs in asthma research

Over the past decade, GWASs have been widely employed to identify risk variants associated with complex diseases. GWASs typically use hundreds of thousands to millions of single-nucleotide polymorphisms (SNPs) as molecular genetic markers to compare the frequency of common genetic variations between individuals and identify phenotype-associated genetic variations through statistical methods.[38] GWASs can help screen high-risk individuals for disease, enhance disease prediction models using genetic information, and ultimately improve patient outcomes through early detection, prevention, or treatment. Commonly used software and packages include: GWAtoolbox,[39] qqman,[40] GenABEL,[41] PLINK,[42] and METAL.[43]

In 2007, Moffatt et al.[25] conducted a GWAS involving 994 patients with childhood-onset asthma and 1243 non-asthmatics, genotyping over 317,000 SNPs. For the first time, they identified susceptibility variants for childhood asthma located in the chromosomal region 17q21. The strong association between the 17q21 region and asthma has been replicated in multiple GWASs involving different ethnicities among both adults and children.[44,45] Subsequent research expanded the focus to the 17q12-21 asthma locus, with orosomucoid like 3 (*ORMDL3*), gasdermin B (*GSDMB*), and post-glycosylphosphatidylinositol (GPI) attachment to proteins phospholipase 3 (PGAP3) being identified as prime candidate asthma genes.[46] A recent asthma GWAS based on the UK Biobank (UKB) analyzed 64,538 asthma cases and 329,321 controls, identifying

145 asthma-associated loci, including 41 newly discovered loci. They further harmonized the results with GWAS summary statistics from the Trans-National Asthma Genetic Consortium (TAGC) and performed a meta-analysis. This comprehensive investigation ultimately identified 66 previously unknown asthma-associated loci and replicated 143 out of 146 previously known genomic regions.[47]

In addition to studying asthma susceptibility, GWAS research can also be used to investigate asthma disease progression and response to drug treatment. Yan et al.[27] conducted a GWAS meta-analysis on 4010 Latino youth with asthma from four independent cohorts, finding that the SNP rs2253681 in FLJ22447 was significantly associated with severe asthma exacerbations. Tantisira et al.[48] identified the SNP rs37972 through GWAS, which was related to the response to inhaled glucocorticoids in asthma. SNP rs37972 was found to be in complete linkage disequilibrium with the glucocorticoid-induced transcript 1 gene (*GLCCI1*), revealing novel pharmacogenetic determinants of the response to inhaled glucocorticoids.

With the acquisition of large-scale GWAS data, significant SNPs can be employed to construct polygenic risk score (PRS) models to estimate the genetic susceptibility of asthma. Namjou et al.[49] used PRS-CS, a Bayesian regression framework method, in the summary statistics from TAGC, to build childhood asthma PRS. The PRS can predict individual disease risk based on genotype, identify high-risk populations, and offer early monitoring and intervention for these groups, providing an effective reference for individualized precision treatment of asthma.

Over the past decade, the development of high-throughput sequencing technologies, increased data availability, and the emergence of high-performance computational tools have greatly advanced GWASs, leading to the discovery of an increasing number of related genetic variations. Despite the identification of hundreds of genetic variations potentially associated with asthma, several challenges still exist in this field. GWASs can only explain a small portion of the overall heritability for asthma.[47,50] Ferreira et al.[51] found that GWAS-identified risk variations explained 25.6% of childhood asthma heritability and 10.6% of adult asthma heritability.

There are several possible explanations for the low proportion of heritability explained by these variations. GWASs only incorporate common variations, not rare variations or copy number variations.[52] WGS and WES can identify disease susceptibility structural variations not captured by GWASs, which potentially could address these issues. Additionally, the impact of a single genetic variation on asthma risk is low.[53] Environmental factors may play a significant role in asthma susceptibility and progression through stable and potentially heritable genomic modifications, which could be investigated in combination with epigenetics.[54] Gene–gene and gene–environment interactions are expected to explain some of the missing genetic contributions.[55] Finally, most asthma GWAS participants are from European populations, and more non-European GWASs or more effective cross-ancestry prediction models are needed to decipher shared genetic variations in asthma susceptibility among different populations.[56] In summary, more asthma risk variations await discovery, requiring larger research samples, more advanced sequencing methods, and more effective statistical models to explore the polygenic architecture of asthma.

Mendelian randomization

Based on the results of GWASs, an increasing number of researchers have recently begun to use Mendelian randomization (MR) to investigate the causal relationship between the exposure of interest and asthma. MR studies use genetic variations (most commonly SNPs) as instrumental variables (IVs) to determine whether the observed association between exposure and disease is likely to be causal.[57] IVs need to meet three assumptions:[1] IVs are associated with exposure (relevance assumption);[2] IVs are related to the outcome only through the studied exposure (exclusion restriction assumption); and[3] IVs must not be related to confounders associated with both the exposure and the outcome (independence assumption).[58] Common MR research methods include inverse variance weighted (IVW), MR-Egger, and weighted median (WM). IVW estimates the causal effect by weighting the IVs based on their inverse variances and works best when all IVs are valid.[59] MR-Egger considers potential horizontal pleiotropy and provides an intercept term to indicate the presence of such pleiotropy.[60] WM considers the median of the weighted IV estimates, which is robust to the presence of invalid genetic instruments.[61] The TwoSampleMR R package is the most commonly used tool for MR analysis.[62]

MR analyses include methods like single-sample MR, two-sample MR, bidirectional MR, two-stage MR, and multivariable MR. Bidirectional MR stands as a more in-depth approach built upon single-sample MR or two-sample MR. It operates by conducting two MR analyses, inverting exposures and outcomes for a given phenotypic pair, which aids in deciphering the directionality of causality.[63] In situations where exposures and outcomes might reciprocally influence each other, their intertwined causal relationships can be analyzed using bidirectional MR. For example, Sun et al.[64] initially used 73 SNPs as IVs to assess the causal effect of body mass index (BMI) on asthma in 56,105 adults through single-sample MR. Following this, a reverse MR analysis was undertaken. The study ultimately ascertained a unidirectional causal link between genetically determined increases in adult BMI and an elevated risk of asthma.

Transcriptomics

Transcriptomics examines the complete set of RNA transcripts produced by an organism's genome. This includes messenger RNA (mRNA), which carries the genetic information from DNA to the ribosomes for protein synthesis, as well as other types of RNA molecules such as non-coding RNA and splice variants.[65] As second-generation sequencing technology has emerged and the cost of sequencing-based technology has decreased, research in transcriptomics has become increasingly popular. This popularity can be attributed to the ability of transcriptomic analysis to provide comprehensive information on gene expression patterns and regulatory mechanisms in various biological systems.

Transcriptomic analysis involves the measurement and quantification of gene expression levels, alternative splicing events, and post-transcriptional modifications. Two primary techniques used for transcriptomic studies are microarray-based and RNA-sequencing (RNA-Seq) based. Statistical models, including differential gene expression (DE) analysis, co-expression network analysis, and pathway analysis, are commonly employed to analyze samples. To increase the power and reproducibility of results, it is common to replicate findings using additional independent datasets by meta-analysis. Commonly used software including DESeq2,[66] limma,[67] edgeR[68] for DE analysis, weighted correlation network analysis (WGCNA)[69] for co-expression network analysis, and Gene Set Enrichment Analysis (GSEA),[70] Ingenuity Pathway Analysis (IPA)[71] for pathway enrichment analysis.

During the research process, confounding factors may affect the analysis. For example, ethnicity and ancestry can be controlled by selecting the first few principal components (PCs). Batch effect introduces biases to the analysis that also need to be addressed. Additionally, the cellular composition of bulk samples can be a confounding factor that affects the interpretation of results. To account for this, cell deconvolution methods are used to adjust for the cellular composition.

Most childhood asthma studies analyze samples from blood, nasal epithelium, or sputum since those are readily accessible and non-invasive ways to study the underlying mechanisms of asthma. Blood samples provide information on immune responses and systemic inflammation. Nasal epithelial samples are useful for studying airway inflammation and epithelial function; some studies have suggested that nasal samples can serve as a good surrogate for lower respiratory samples in the study

of lung diseases. Sputum samples contain a mixture of mucus, saliva, and cells from the lower respiratory tract that provide a direct representation of the airway secretions and cellular components that are present in the lungs. More invasive sampling methods such as bronchoalveolar lavage (BAL)[72,73] and endobronchial biopsy,[74] both of which require bronchoscopy, have also been employed in asthma transcriptomic research. During a BAL, saline is instilled through the bronchoscope into a lobe of the lung then collected via suctioning for analysis, while endobronchial biopsies involve taking small samples of tissue from the airway lining. Detailed studies can be referenced in a review.[75]

Th2/Th17 asthma subtypes

Asthma is caused by diverse pathogenic mechanisms which contribute to the variability in clinical presentation and treatment response. Th2 and Th17 immune pathways are important in the development and progression of asthma and are considered key endotypes of the disease. Recently, transcriptomic analysis has been increasingly used to investigate the biological mechanisms underlying the Th2 and Th17 endotypes of asthma. This approach has yielded valuable insights into the gene expression patterns and signaling pathways involved in these immune pathways and has helped to identify potential therapeutic targets for asthma treatment.

Th2-driven inflammation is a type of immune response that is characterized by the activation of Th2 cells and the production of cytokines such as interleukin IL-4, IL-5, and IL-13.[76] In 2006, Woodruff et al.[77] first defined the three Th2 gene markers, chloride channel, calcium-activated, family member 1 (*CLCA1*), serine peptidase inhibitor, clade B (ovalbumin), member 2 (*SERPINB2*), and periostin (*POSTN*), by using microarray from airway epithelial samples. The three genes are highly expressed in asthma compared to controls and are downregulated by corticosteroid treatment. The presence of three Th2 markers was confirmed by assessing 27 endobronchial biopsy samples.[78] The Th2-high and Th2-low subtypes exhibit contrasting features in relation to eosinophilic inflammation, mucin composition, subepithelial fibrosis, and corticosteroid sensitivity. A similar gene expression pattern was also found by examination of sputum samples obtained from 104 participants afflicted with moderate-to-severe asthma and 16 healthy volunteers.[79] The Th2 endotype was identified in these studies, and it was established that it correlates with elevated eosinophil counts, fractional exhaled nitric oxide (FeNO) levels, and IgE levels.

Th17 cells are a distinctive subset of $CD4^+$ T helper (Th) cells that can induce neutrophilic inflammation by producing interleukin-17 (IL-17), interleukin-22 (IL-22), and interleukin-6 (IL-6).[80] In 2008, McKinley et al.[81] demonstrated the potential involvement of Th17 cells in steroid-resistant asthma using a mouse model. The IL-17 pathway has been associated with the expression of CXC chemokines, granulocyte colony-stimulating factor (GCSF), and IL-6. IL-17A and IL-17F have been shown to upregulate the expression of colony stimulating factor 3 (CSF3), a hematopoietic factor that promotes neutrophil production, and the chemokines chemokine (C-X-C motif) ligand (CXCL) 1, CXCL2, CXCL3, and IL8, which attract neutrophils. These molecules were first used as a marker panel for Th17 cells. Additionally, a significant negative correlation between Th2 and Th17 gene expression has been established. This reciprocal suppression was observed in bronchial epithelial cells, where the Th2 signature transcripts were modestly suppressed by IL-17A + TNF-α, and Th17 signature transcripts were suppressed by IL-13. It is noteworthy that Th2 and Th17 subtypes are mutually exclusive in many studies,[73,82,83] but eosinophilic inflammation has been detected in both Th2-high and Th17-high asthma.[83]

ILC2 asthma subtypes

A relatively new asthma endotype, known as the ILC2 (type 2 innate lymphoid cells) asthma endotype, is garnering increasing attention from researchers.[84] Innate lymphoid cells (ILCs) generate numerous cytokines commonly associated with Th cells, yet they lack the cell surface markers linked to other immune cell lineages (referred to as lineage-negative or Lin−) and do not possess a T-cell receptor (TCR). In response to allergic reactions, ILC2s rapidly produce a variety of type 2 cytokines such as IL-4, IL-5, IL-13, IL-9, and amphiregulin (Areg). They also engage in intercellular communication to regulate immune responses. In the context of asthma, when activated by alarm cytokines released by epithelial cells, ILC2s contribute to pulmonary inflammation by secreting type 2 cytokines like IL-4, IL-5, and IL-13.[85] These cytokines further exacerbate asthma symptoms by enhancing smooth muscle contraction, mucus production, and the recruitment of inflammatory cells in the lungs.[86] Currently, there are some challenges in the research related to ILC2. Unlike some other immune cells, ILC2s do not have widely recognized surface markers, making their identification and analysis relatively challenging. Furthermore, although there has been some progress in the study of ILC2s, there is relatively limited research in the field of transcriptomics.

In summary, transcriptomics continues to evolve, and its integration with other omics disciplines holds promise for uncovering the molecular mechanisms of asthma and advancing personalized medicine approaches.

Epigenomics

Epigenomics is a field of study that explores the various chemical modifications and molecular processes that influence gene expression and regulation. These modifications, known as epigenomic variations, have the ability to modulate gene expression without altering the DNA sequence itself. They are specific to particular cell types and tissues and can respond to various environmental exposures.[87] Examples of these modifications include DNA methylation and histone modifications. Epigenomics aims to understand how these modifications control gene activity and play a crucial role in a wide range of biological processes, including development, aging, and environment-related asthma pathogenesis.[88] By deciphering the epigenetic landscape, researchers can gain valuable insights into the mechanisms underlying gene regulation and potentially uncover novel insights into the pathogenesis of childhood and adulthood asthma.

DNA methylation is a fundamental epigenetic modification that involves the addition of a methyl group to the cytosine at position 5′ in cytosine–phosphate–guanine (CpG) sites.[89] DNA methylation plays a crucial role in gene regulation by influencing gene expression patterns. Generally, high levels of DNA methylation in promoter regions are associated with gene silencing, preventing the binding of transcription factors and other regulatory proteins to the DNA. In contrast, lower levels of DNA methylation, particularly in gene body regions, are associated with gene activation.[90]

DNA methylation is commonly assessed using DNA methylation chips, such as the Infinium Human Methylation 450K beadchip, which covers around 450,000 CpG sites, or the Infinium Methylation EPIC Beadchip, which extends the coverage to approximately 850,000 CpG sites across the entire genome.[91] Bisulfite pyrosequencing is a sequencing-based technique commonly used to analyze DNA methylation patterns at single-nucleotide resolution.[92] Although bisulfite pyrosequencing provides high-resolution methylation data, it is a more costly approach compared to microarray-based technologies. Consequently, the majority of epigenomics analyses have been performed using array-based methods due to their cost-effectiveness.

In statistical analysis, the primary data format is the Beta-value, which represents the ratio of the methylated probe intensity to the overall intensity (sum of methylated and unmethylated probe intensities). Another commonly used data format is the *M*-value, calculated as the log2 ratio of the intensities of the methylated probe compared to the unmethylated probe. The Beta-value method provides a direct biological interpretation, as it represents the proportion of

methylation at a specific CpG site. On the other hand, the *M*-value method is more statistically valid, particularly in differential and other statistical analyses, because it exhibits approximately homoscedastic properties.[93]

EWASs serve as a key analysis structure in the field of epigenomics.[94] These studies aim to identify associations between epigenetic variations and diseases or phenotypes of interest. Additionally, differential DNA methylation region analysis helps identify regions of the genome where DNA methylation patterns differ significantly between different groups or conditions.[95] These regions are referred to as differentially methylated regions (DMRs). DMRs often correspond to regulatory regions, such as promoters or enhancers, and their differential methylation can influence gene expression and cellular processes. Similarly, meta-analysis among multiple studies is usually considered for consolidating findings. Popular statistical packages include Minfi,[96] limma,[67] and methylKit.[97]

As with genome studies, samples are usually from blood, airway, or sputum. Yang and Schwartz[98] conducted a study using blood samples and identified DMRs in genes such as *IL13*, *IL4*, and *RUNX3*, which are well-established to be associated with asthma and atopy. Xu et al.[30] identified hypomethylated whole-blood DNA CpG sites on genes involved in activating eosinophils and cytotoxic T cells. In a meta-analysis of eight cohorts of newborns, Reese et al.[99] identified 9 specific CpG sites and 35 genomic regions that have implications for the development of asthma. Hoang et al.[100] found hundreds of differently methylated CpG sites in blood among adults with non-atopic or atopic asthma compared to those without asthma or atopy. Blood methylation patterns also proved to be a useful proxy for studying atopic asthma in nasal tissue, with practical research implications. Herrera-Luis et al.[101] found consistent DNA methylation patterns in the blood associated with lung function in pediatric asthma among Mexican Americans and Puerto Ricans. These patterns revealed population-specific associations shared among different Latino subgroups. Recto et al.[102] identified 490 statistically significant differentially methylated CpGs associated with IgE in adult blood samples. Thürmann et al.[103] found 158 DMRs in children with asthma compared to controls, and 37% of these DMRs were associated with eosinophil content. Their study unveiled a global hypomethylation pattern predominantly impacting enhancer regions responsible for regulating immune genes such as *IL4*, *IL5RA*, and *EPX*. This highlights the dysregulation of enhancer regions as a distinctive feature of childhood asthma. In nasal epithelial tissue, an EWAS discovered several DMRs associated with asthma and atopy-related genes including *ALOX15*, *CAPN14*, *HNMT*, and *POSTN* using nasal brushings.[104] In a nasal epithelial EWAS meta-analysis, Yan et al.[32] identified 12 genes that exhibited methylated CpG sites associated with exposure to violence and chronic stress. Furthermore, these genes were linked to childhood atopic asthma. An EWAS of Puerto Rico children with atopic asthma identified differentially methylated genes relevant to epithelial barrier function, airway epithelial integrity, and immune regulation.[105]

Future directions include single-cell epigenomics, multi-omics integration, environmental epigenomics, and epigenetic-based therapies, offering promising insights into asthma's molecular mechanisms and personalized treatments.

Microbiome and metagenomics

While asthma has traditionally been considered primarily driven by immune responses to allergens and irritants, emerging evidence suggests that the respiratory microbiome may play a crucial role in asthma pathogenesis and exacerbation.[106–108] The microbiome encompasses a diverse community of microorganisms residing within the human body, including bacteria, viruses, and fungi. Metagenomics, defined as the study of genetic material extracted directly from environmental samples, involves a comprehensive analysis of this genetic material, enabling the identification and characterization of various microorganisms without the need for cultivation.[109,110] Researchers have increasingly recognized the potential influence of the microbiome on the development, progression, and exacerbation of the disease. Alterations in the composition and diversity of the airway microbiota have been linked to airway inflammation and immune dysregulation observed in individuals with asthma.[106,111–114]

Common quantification methods include 16S, 18S, and Whole Metagenome Shotgun Sequencing (WMGS). 16S sequencing targets a conserved region of the 16S ribosomal RNA gene, a genetic marker found in bacteria and archaea.[115] On the other hand, 18S targets the 18S ribosomal RNA gene, commonly found in eukaryotic microorganisms like fungi and protists, enabling researchers to assess the diversity of these eukaryotic organisms.[116] WMGS involves sequencing all the genetic material present in a sample, including both microbial and host DNA, providing a comprehensive view of the genetic diversity and functional potential of all microorganisms present. Most asthma studies have focused on the gut and respiratory tract samples, including BAL, sputum, bronchial biopsies, or nasal swabs/lavage. Extensive research has demonstrated that an imbalance in the gut microbiota, known as dysbiosis, significantly enhances susceptibility to asthma. This phenomenon, termed the "gut–lung axis," underscores the pivotal role of the gut microbiome in the realm of respiratory ailments.[117]

These microbiome studies, challenging traditional views that focused solely on immune responses, hold the potential to uncover novel biomarkers, therapeutic targets, and interventions that could better manage and treat asthma. Further research is needed to unravel the intricate interactions between the respiratory microbiome, host immune responses, and the pathogenesis of asthma, ultimately leading to improved diagnostic and therapeutic strategies for this complex respiratory condition.

Integrative omics analysis

The integration of multi-omics sequencing data provides a comprehensive framework for understanding the molecular regulatory mechanisms of asthma. As is shown in Fig. 2, starting from the identification of genetic variations like SNPs, we delve deeper into their functional implications. Expression quantitative trait loci (eQTL) has been developed to explore the associations between genomics and transcriptomics,[29] while methylation quantitative trait loci (mQTL) associates these SNPs to specific methylation patterns. One particularly revealing approach within this context is expression quantitative trait methylation (eQTM), which associates methylation patterns with transcript levels.

In a previous study we conducted, eQTL analysis was employed to examine genomic and transcriptomic associations in whole blood from 121 Puerto Rican children. This study led to the discovery of several asthma-related genes influenced by specific genetic variations, such as *SCGB3A1*, *IPO8*, *CHURC1*, and *FAM118A*.[118] By connecting genomics to transcriptomics, researchers can gain deeper insights into how genetic variations influence gene expression, potentially unraveling the molecular mechanisms driving the complex disease process of asthma.[119] Similarly, mQTL analysis can be used to identify genetic loci associated with site-specific DNA methylation of CpGs.[120]

Parallelly, eQTM studies can be used to explore the distant epigenetic regulation of gene expression in asthma.[121] eQTM refers to the association between DNA methylation patterns and gene expression levels. It aims to identify DNA methylation sites (often CpG sites) that are correlated with gene expression levels and provide insights into the regulatory mechanisms underlying gene expression directly. There are two main types of eQTM analysis, defined by the gene–CpGs distance. *Cis*-eQTM refers to the association between DNA methylation at genetic loci near a gene. It focuses on DNA methylation sites within the vicinity of the target gene, typically within a certain genomic distance, such as the promoter region or nearby *cis*-regulatory elements. In contrast, *trans*-

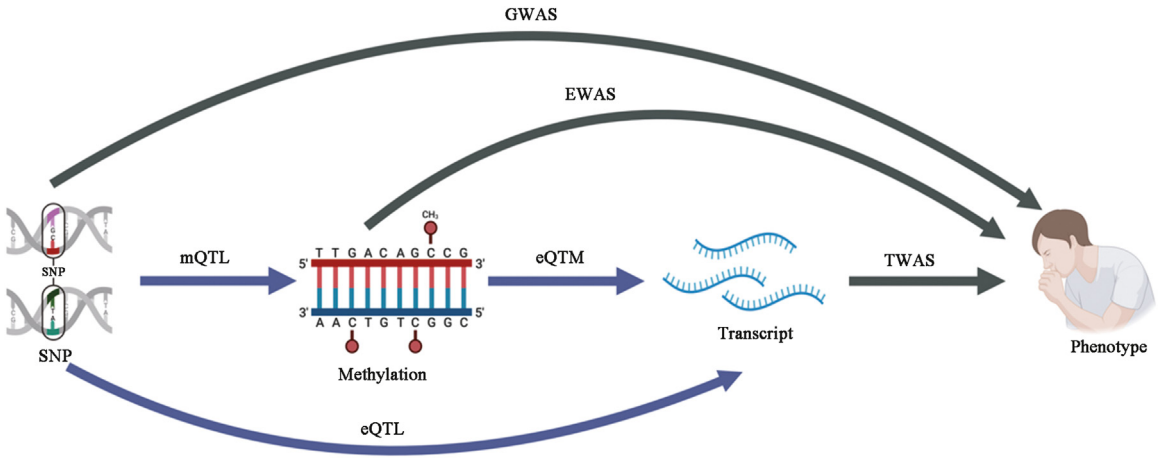

Fig. 2. Multi-omics integration enables a comprehensive understanding of asthma. Figure was created with Biorender.com. eQTL: Expression quantitative trait loci; eQTM: Expression quantitative trait methylation; EWAS: Epigenome-wide association study; GWAS: Genome-wide association study; mQTL: Methylation quantitative trait loci; SNP: Single-nucleotide polymorphism; TWAS: Transcriptome-wide association study.

eQTM examines the relationship between DNA methylation at genetic loci distant from a gene and the expression of that gene. *Trans*-eQTM analysis identifies associations between DNA methylation and gene expression that occur across chromosomes.

Kim et al.[121] utilized nasal epithelium samples and identified a total of 16,867 significant pairs of methylation-gene expression associations. Notably, the majority of these associations were characterized as *trans*-eQTM signals. They also identified 5934 paths that represent potential pathways connecting the effects of methylation markers on gene expression to the development of atopic asthma through mediation analysis.

To further integrate multiple layers of information, colocalization studies can be utilized to integrate multi-omics data, such as combining GWAS with eQTL and mQTL, to investigate the regulatory effects of asthma risk variants.[122] These approaches hold promise for providing new insights into the regulatory mechanisms of asthma.

Summary and prospects

As the field of asthma omics research continues to advance rapidly, there are several promising directions for future exploration. To our knowledge, most of the current asthma omics research is based on bulk sequencing, such as bulk RNA-seq, which detects the average expression level of genes at the tissue level, thereby masking cellular heterogeneity. Single-cell sequencing offers the possibility of identifying molecular variances exclusively associated with specific cell types, enabling the detection of rare cell subsets and key cellular processes involved in disease development, which is crucial for studying the mechanisms of asthma.[123] For example, Tibbitt et al.[124] conducted single-cell RNA sequencing (scRNA-seq) on Th cells isolated from BAL samples in mouse models of allergic airway inflammation and identified previously undescribed Th cell subpopulation. Liu et al[125] conducted scRNA-seq analysis on mouse lung immune cells and identified Creb5 and CD11b-DCs as regulatory factors in asthma exacerbation. These studies demonstrate the successful application of single-cell sequencing in asthma research.

Moreover, recent developments in single-cell omics technologies such as CITE-seq (cellular indexing of transcriptomes and epitopes by sequencing)[126] and DOGMA-seq, an adaptation of CITE-seq for measuring gene activity across the central dogma of gene regulation,[127] enable multi-modalities measurements for the same cell at the genome, transcriptome, or epigenome scale, which are anticipated to provide unprecedented insight and resolution for asthma research. In addition, the emerging spatial transcriptomics technology, capable of providing whole transcriptome data with spatial information, offers opportunities for investigating the spatial location of asthma immune cells in the future.[128,129]

Table 1 presents a curated list of publicly available genomics datasets related to asthma. These datasets serve as vital foundations for scientists, clinicians, and researchers worldwide, offering valuable information for us to deepen our understanding of this chronic respiratory condition. Researchers can leverage these resources to identify novel biomarkers, unveil intricate disease mechanisms, and develop personalized treatment strategies. Although advancements in high-throughput sequencing technologies have generated increasingly high-dimensional multi-omics data, the integration of multi-omics analyses in asthma research remains in the primary stage. The future challenge will involve analyzing and integrating these multi-omics datasets, requiring more efforts to develop various multi-omics integration analysis methods, including machine learning and deep learning approaches, to gain deeper and more comprehensive insights into the molecular processes underlying asthma pathogenesis and progression.[75,130]

Omics has immense potential to become a precision medicine tool used in the clinical care of individuals with asthma. Despite the abundance of knowledge derived from asthma omics research, asthma omics approaches are not yet routinely employed in the clinical setting.[75,131] For omics testing to translate to clinical practice, ideally, testing should be inexpensive, non-invasive, readily available, and indicated for specific clinical questions (e.g., for asthma diagnosis, phenotyping, monitoring, or therapeutic decision-making), and results should be simple to interpret in the context of the clinical question and should impact medical decision-making. Because asthma control fluctuates in an individual patient, short wait time for results would also greatly enhance the feasibility of employing omics testing for disease monitoring or therapeutic decision-making. High sensitivity, specificity, accuracy, and precision are also ideal qualities of tests routinely employed in clinical practice. Further research will be needed to establish which specific omics approaches can and should be applied in the routine clinical care of individuals with asthma.

In sum, our understanding of asthma, a highly complex and prevalent disease, has been greatly enhanced through omics techniques examining the genome, transcriptome, and epigenome. Ongoing omics research, including studies that utilize emerging technologies such as scRNA and multi-omics approaches, has immense potential to further elucidate valuable information on asthma pathogenesis and progression.

Table 1
Public available genomics datasets of asthma.

Study	Data type	Sample	Sample Size	GSE/EGA ID
Genes-environments & Admixture in Latino Americans (GALAII)	Gene expression (RNA-seq)	Nasal brushes	695	GSE152004
University of Chicago Asthma & COPD Center	Gene expression (RNA-seq)	Airway epithelial cells (AECs)	85	GSE85568
Prevention and Incidence of Asthma and Mite Allergy (PIAMA)	Gene expression (RNA-seq)	Nasal brushes	186	EGAD00001008767
Chronic Rhinosinusitis Integrative Studies Program (CRISP)	Gene expression (RNA-seq)	AECs	190	GSE172367
Severe Asthma Research Program (SARP)	Gene expression (Microarray)	Bronchial biopsy	108	GSE43696
Mechanistic Indicators of Asthma (MICA)	Gene expression (Microarray)	Peripheral blood	131	GSE35571
AllerGen Clinical Investigator Collaborative (CIC)	Gene expression (Microarray)	Peripheral blood	28	GSE40240
GSE41649	Gene expression (Microarray)	Bronchial biopsy	8	GSE41649
The Nationwide Study on Problematic Severe Asthma in Sweden	Gene expression (Microarray)	White blood cells	54	GSE27011
GSE18965	Gene expression (Microarray)	AECs	112	GSE18965
GSE16032	Gene expression (Microarray)	Peripheral blood mononuclear cells (PBMCs)	50	GSE16032
Mechanisms of Acute Viral Respiratory Infection in Children (MAVRIC)	Gene expression (Microarray)	Nasal swab specimen	106	GSE103166
The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED)	Gene expression (Microarray)	Blood	216	GSE123750
Inner City Asthma Consortium (ICAC)	Gene expression (Microarray)	PBMCs	194	GSE40736
Swedish Abbreviation for Children, Allergy, Milieu, Stockholm, Epidemiology (BAMSE)	Gene expression (Microarray)	Whole blood	256	GSE141623
INfancia y Medio Ambiente (Environment and Childhood) (INMA)	Gene expression (Microarray)	Whole blood	201	GSE141631
Inner City Asthma Consortium (ICAC)	DNA methylation (Microarray)	PBMCs	194	GSE40736
University of Chicago Asthma & COPD Center	DNA methylation (Microarray)	AECs	115	GSE85568
GSE109446	DNA methylation (Microarray)	Nasal epithelial cells (NECs)	29	GSE109446
Chronic Rhinosinusitis Integrative Studies Program (CRISP)	DNA methylation (Microarray)	AECs	206	GSE172365
Genes-environments & Admixture in Latino Americans (GALAII)	DNA methylation (Microarray)	Whole blood	573	GSE77716
Prevention and Incidence of Asthma and Mite Allergy (PIAMA)	DNA methylation (Microarray)	Nasal brushes	696	EGAD00010002263

COPD: Chronic obstructive pulmonary disease; RNA-seq: RNA-sequencing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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