Review

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Review

Genetic, Inflammatory and Immunological Biomarkers for the Early Assessment of Allergic Asthma: A Current Update

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Abstract: Asthma is a complex syndrome with multiple phenotypes and endotypes, and current diagnostic tools often fail to address this, therefore there is an increasing need for new predicting biomarkers for asthma which can enable personalized treatment strategies. Biomarkers can facilitate earlier diagnosis of asthma, potentially identifying the disease before clinical symptoms appear. This allows for early intervention, which can prevent asthma progression and complications and improve long-term outcomes. Reliable biomarkers can help in monitoring disease activity and treatment efficacy in real-time and can also lead to better disease management, reducing hospitalizations, emergency visits, and overall healthcare costs associated with poorly controlled asthma. Severe asthma patients often do not respond well to standard treatments. New biomarkers can help identify these patients and guide the development of novel therapeutic approaches specifically for severe asthma. In this review we summarize different candidate biomarkers on several levels of investigation: Genetic, subclinical inflammatory, allergen-specific immune response, and trained immunity, which could be promising for the early detection of asthma and better management of exacerbation and severity of symptoms in children.

Keywords: asthma; biomarker; allergen-specific; subclinical; exacerbation

1. Introduction

Asthma is a prevalent chronic respiratory condition that affects people of all ages, including young children. For long-term complications to be effectively managed and avoided, early assessment of childhood asthma is essential [1]. Due to the lack of specific symptoms and the difficulties in differentiating asthma from other respiratory illnesses, it can be challenging to diagnose asthma in children at an early age, especially when distinguishing between children who have real asthma and those who have temporary wheezing linked to viral infection [2].

It is essential to take immunological, inflammation, and genetic biomarkers into account for the early assessment of childhood asthma. Research has indicated that some genetic markers are linked to a higher likelihood of developing asthma throughout early life. Furthermore, the onset and severity of childhood asthma have been related to immunological indicators such as increased levels of certain cytokines [3]. Personalized treatment approaches and tailored screening tactics require an understanding of the function these indicators play in the early diagnosis of asthma. The diagnosis of asthma in early infancy can be more accurate by utilizing genetic and immunological indicators. This would enable prompt treatments to prevent long-term consequences and ensure that children with asthma receive the right management strategies.

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2. Genetic Biomarkers for Asthma

2.1. Single Nucleotide Polymorphisms of 17 Q21 Chromosome

In 1997 the Collaborative Study on the Genetics of Asthma (CSGA) was published as the first scientific work to examine the heterogeneity of asthma. Researching the genetic variants and polymorphisms in genes related to immunological response and airway inflammation revealed multiple potential genes that could be crucial in the development of asthma. These potential genes include the IL-4 receptor, A disintegrin and metalloproteinase 33 (ADAM33), and ORMDL Sphingolipid Biosynthesis Regulator 3 (ORMDL3). Furthermore, single nucleotide polymorphisms linked to an increased risk of developing asthma in early childhood have been discovered by genome-wide association studies. Subsequent research has demonstrated a specific correlation between ORMDL3 gene variants and an elevated risk of asthma in early childhood [4].

The first asthma susceptibility gene to be positionally cloned was ADAM33, which was initially discovered in 2002 and was shown to be strongly linked both to asthma and bronchial hyperresponsiveness. The gene's precise location in the genome was determined before its function was understood [5]. ADAM33 is located on chromosome 20 and encodes a protein involved in airway remodeling and smooth muscle cell proliferation and processes that contribute to the development of airway obstruction in asthma [6].

The ORMDL3 gene, located on chromosome 17, is also involved in regulating airway inflammation and bronchial hyperresponsiveness. Understanding the specific genetic variations within the ORMDL3 gene and their impact on asthma susceptibility is critical for refining early detection and intervention strategies [7].

A thorough analysis reviewed the findings of 42 GWASs (genome-wide association studies) on asthma different phenotypes was published in 2019. This study summarizes the numerous risk alleles and loci that were replicated in several populations. 17q12–21 has been the most often replicated asthma locus in GWASs, followed by 6p21 (HLA region), 5q22 (TSLP), 2q12 (IL1RL1/IL18R1), and 9p24 (IL33) [8].

Similarly, the Interleukin-4 receptor gene, which plays a central role in immune responses, has been linked to the regulation of allergic inflammation and the development of asthma symptoms in children. According to a previous meta-analysis, IL4 gene -589C/T polymorphism was found to be a susceptibility risk for asthma in adults and pediatrics across all ethnic groups [9].

2.2. Micro RNAs (miRNAs)

miRNAs, which consist of 18-25 nucleotides, are short single-stranded non-coding RNAs and play an essential role in regulating gene expression and gene networks. Direct binding of miRNA to a mRNA may lead to either mRNA degradation or translational repression of the target mRNA. Recent studies of miRNAs in asthma and allergies has revealed a large number of miRNAs that could help diagnose and understand the condition. In a mouse model of asthma induced by ovalbumin (OVA), MiR-221 inhibition reduced the airway inflammation linked to asthma. It has been suggested that miR-221 stimulates the release of cytokines and mast cell degranulation [10]. miR-21, miR-145 and miR-126 were also well-studied in mice. It was shown that the inhibition of those RNAs in mice resulted in reduced allergic inflammation, mucus excretion, Th2 cytokine production (IL-5 and IL-13), and airway hyperreactivity [11]. In contrast, another study revealed that miR-18a, miR-27a, miR-128, and miR-155 were down-regulated in asthmatic human bronchial epithelial cells and that the inhibition of these miRNAs results in a significant elevation of IL-8 and IL-6 [12]. Certain miRNA modules found in sputum, such as miR-223-3p, to TLR/Th17 signaling, were linked to lung function impairment, hospitalizations, and the frequency of neutrophils in sputum. MiR-629-3p, derived from epithelial cells, plus miR-223-3p and miR-142-3p, derived from neutrophils, were increased in sputum from severe asthmatics [13]. These results show that mRNAs could be promising biomarkers in asthma early assessment and management.

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3. Subclinical Inflammation Biomarkers for Asthma

3.1. Eosinophil-Derived Neurotoxin (EDN) RNase 2 and Eosinophil Cationic Protein (ECP) Rnase 3

When eosinophils are activated, a protein called EDN is released from their granules.

EDN has shown promise as a biomarker for eosinophilic inflammation in asthma and wheezing symptoms in children. When EDN was investigated as a biomarker for asthma exacerbations in children, the level of the biomarker was a strong predictor of the length of hospital stay. EDN has demonstrated potential as an asthma predictor and has been used successfully in clinical trials to evaluate the responsiveness to asthma treatment [14].

Eosinophil cationic protein (ECP) is also released from eosinophils and was studied for a long time as a potential asthma biomarker until researchers found that EDN was more promising. Both EDN and ECP belong to the same subfamily of ribonuclease (RNase) multigenes expressed in eosinophils. They share 66% of their amino acid sequence and are released almost exclusively by eosinophils, which makes them good biomarkers for eosinophil activity, they also carry out similar roles in pathophysiology, including ribonuclease activity and toxicity toward parasites [15].

In terms of test reproducibility EDN is better than ECP. Due to its lower electrical charge, EDN may be retrieved from measuring devices and cell surfaces more readily [16]. Additionally, EDN is known to be released from eosinophils more efficiently than ECP, which increases its recoverability and supports a potential pathophysiological role. EDN, in contrast to ECP, exhibits antiviral efficacy against respiratory infections that are strongly linked to the development of allergy diseases later in life (e.g., early childhood infections with RSV and human rhinovirus. Serum EDN level was more strongly correlated with symptom severity score than serum ECP level or total eosinophil count in young children [17].

3.2. High-Sensitive C- Reactive Protein (hsCRP)

High-sensitive assays of CRP (hs-CRP) allow detection of CRP levels that were previously undetectable using normal tests. Previous studies revealed the correlation between elevated serum hs-CRP and low forced expiratory volume in one second (FEV1) and airway hyperresponsiveness, indicating a potential link between systemic inflammation and respiratory impairment. High-sensitivity C-reactive protein (hs-CRP) is a marker of low-grade systemic inflammation, which has been closely linked to many non-communicable diseases (NCD), and childhood allergic diseases are considered the earliest debuting NCD [18]. A prior study including 150 children with asthma demonstrated a strong correlation (p=0.019) between hs-CRP and FEV1%. The area under the curve for hs-CRP mg/L to distinguish between uncontrolled and (controlled + partly controlled) asthma was 0.67 (95% CI 0.55, 0.80) using Receiver Operator Characteristic (ROC) analysis, and a cutoff 1.1 mg/L of serum hs-CRP level showed a sensitivity of 68.1% with a specificity of 67.97%. Higher serum hs-CRP levels in uncontrolled asthma were observed, supporting the use of hs-CRP as a substitute marker for small airway inflammation [19]. In the following table, we summarize the cutoff, sensitivity, and specificity of hs-CRP related to asthma in several previous studies.

Table 1. Cutoff, sensitivity and specificity of hs-CRP related to asthma in several previous studies.

Author	Cutoff	ROC/ AUC	Sensitivity	Specificity	P value
(Kumar, 2023) [19]	1.1 mg/L	0.67 (95% CI 0.55, 0.80)	68.1%	67.97%	p = 0.03
(Monadi, 2016) [20]	1.45 mg/L	0.635±0.037	accuracy of 63.5 %		p =0.001
(Deraz, 2011) [21]	2.1 mg/L		72%	93%	0.0001

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3.3. Nitric Oxide Synthetase

It has been proposed that fractional exhaled nitric oxide (FeNO) is a noninvasive indicator of airway inflammation. Nitric oxide (NO) levels in exhaled air can be tested using a continuous expiratory flow breathing technique, which allows for a standardized measurement of FeNO. FeNO is used nowadays to diagnose asthma, predict a patient's reaction to inhaled steroids, evaluate a patient's response to inhaled steroids, determine the best dose for controlling asthma, and act as a relevant indicator for biological medicaments. Patients with type 2 inflammation and asthma, as well as those with allergic rhinitis and chronic rhinosinusitis with nasal polyposis, exhale higher levels of FeNO. Furthermore, exposure to mold, moisture, and allergens raises FeNO, which is significantly decreased by steroid treatment [22]. Nitric oxide (NO) is generated by converting L-arginine to L-citrulline through one of three different isoforms of NO synthases (NOSs). These isoforms are neuronal NOS (also known as NOS1), endothelial NOS (also known as NOS3), and inducible NOS (also known as NOS2). In the lungs, NOS2 produces the most NO during inflammation, while NOS1 and NOS3 each produce minimal amounts of NO. When inflammatory stimuli such cytokines, oxidants, viral infections, and allergen exposure occur, human airway nerves produce NOS1, endothelial cells of the pulmonary circulation produce NOS3, and T cells, macrophages, epithelial cells, mast cells, eosinophils, and neutrophils produce NOS2 [23].

The most important source of NO is produced by iNOS, which is typically activated by immunological or inflammatory stimuli such cytokines. Additionally, during airway inflammation, iNOS is mostly produced in macrophages and epithelial cells found in the airway epithelial cells of asthmatic patients. The increased expression and activity of iNOS enzyme cause elevated NO levels in FeNO in asthmatic patients. Uncertainty surrounds the pathophysiological significance of elevated iNOS in pediatric allergic asthma and rhinitis. iNOS was elevated among the decreased lung function group and may serve as a biomarker for the development of asthma and reduced lung function. Elevated levels of IL-1β and iNOS in the serum were considered significant indicators of the likelihood of comorbidity of allergic rhinitis and asthma in children. [24]. Asthma patients are known to have elevated amounts of exhaled NO, and it is well-recognized that iNOS is upregulated in their lungs. In both normal and asthmatic patients, more oral or inhaled L-arginine increases exhaled NO, suggesting that the bioavailability of L-arginine for NOS determines the generation of NO in the airways of both healthy and asthmatic subjects [25].

3.4. Arginase

Another important enzyme involved in the nitric oxide pathway and related to asthma is arginase, which controls the bioavailability of L- arginine. Arginase has a crucial role in the pathophysiology of experimental asthma, according to studies on human asthma. It took decades for the role of elevated arginase activity in the sputum of asthmatic patients to be further clarified, even though this was noted as early as 1980 [26].

Focus has lately shifted to the role that arginase's pathway of L-arginine catabolism plays in controlling the synthesis of endogenous NO, and how this affects airway function in lung conditions like asthma. L-arginine is degraded to produce urea and L-ornithine by the urea cycle enzyme arginase. The hepatic cells' cytoplasm contains both the constitutively produced forms of arginase I and II, whereas the extrahepatic and mitochondrial forms of arginase I. Airway hyperresponsiveness during the early asthmatic reaction and late asthmatic reaction may be caused by increased arginase activity since this enzyme regulates the bioavailability of L-arginine for NOS through competitive consumption of the substrate. Arginase activity is elevated in the airways of allergen-challenged mice concurrently with a decrease in L-arginine and L-citrulline levels [27].

Asthma patients' bronchoalveolar lavage samples and airway epithelium from bronchial biopsies have now been shown to exhibit increased enzyme activity, mRNA, and protein expression arginase I [28].

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4. Allergen-Specific Immune Response Biomarkers

4.1. Allergen-Specific IgE Antibodies

The function of immunoglobulin E (IgE) in allergic reactions is well-known. It is produced by plasma cells and capable of precisely identifying allergens and generating an immune response. When an allergen is recognized again, the immune system becomes sensitized, releasing different chemokines and cytokines that cause symptoms of atopic diseases such as bronchospasm in asthma, mucous hypersecretion in rhinitis, and local inflammation in eczema [29].

Elevated total serum IgE indicates a high possibility of the presence of atopic diseases in children with allergy-like symptoms. Nevertheless, the longitudinal trends of total serum IgE levels and their association with allergen sensitization and atopic diseases during early childhood are still lacking.

Patients with allergic disorders typically have increased levels of total serum IgE and it has been proposed that total IgE can be used to predict the onset of allergy diseases. Early childhood atopic disorders and allergen sensitization may be predicted by blood total IgE levels. Serum total IgE levels higher than 100 kU/L in infants may indicate the existence of food allergy, whereas levels higher than 200 kU/L may suggest a high risk of eczema [30]. Serum total IgE level higher than 200 kU/L is correlated to mite sensitization in young children (after the age of 2), and children with high house dust mite-specific IgE level may be at significant risk for allergic rhinitis and asthma. Therefore, eczema in infants and rhinitis and asthma in later life are most likely to occur when serum total IgE levels are higher than 200 kU/L from infancy to early childhood, indicating the need for early diagnosis biomarkers to detect childhood atopic diseases [31].

Several methods were used to detect allergen specific antibodies: The first applied technique is synthetic peptide array technology assay membrane, which is now less utilized due to its inability to quantify epitope binding, the high volume of patient serum required, and the membrane's limit on number of epitope–IgE interactions [32]. Another method is capture ELISA which is useful for IgE detection to whole protein or epitope peptides, but not well standardized and time-consuming [33].

Peptide microarray has been well studied to identify epitope-specific antibodies in several allergies including egg, milk, and fish allergens, and has the advantage of utilizing small amounts of patient samples, but requires advanced tools for bioinformatics [34]. Compared to peptide microarray tests, bead-based epitope assay (BBEA) offers a higher sensitivity of epitope-specific IgE detection and is highly reproducible, accurate, and less time-consuming. The assay enables the simultaneous detection of up to 94 patient samples with 100 different epitope-specific IgE antibodies, and the technique is comparatively easy to perform. The limitations of this method are that the data processing bioinformatics experience, and the binding signal is presented in median fluorescence intensity units (not in Ius/mL), which is not currently utilized in clinical practice [35].

4.2. Allergen-Specific T- Cells

Asthma and allergy disorders have been more frequent in the industrialized world over the past few decades, which has led to studies into the underlying immunologic and environmental mechanisms causing this common disease. Since the distinction between T Helper 1 and T Helper 2 immune responses was initially clarified, it was determined that Th2 cells play an essential role in allergic asthma. Prescott et al. discovered that atopy persistence in early childhood may be attributed to a combination of impaired Th1 immune responses and allergen-specific Th2 immune responses [36].

The involvement of additional T helper subsets in asthma has also been clarified: aberrant Th17 or Th2/Th17 immune responses, which stimulate IL-17A production and airway neutrophilia; Th9 immune responses, which stimulate IL-9 production, facilitating mast cell proliferation and activation as well as airway remodeling; and impaired regulatory responses because of insufficient production of immunoregulatory cytokines (e.g., transforming growth factor-β and IL-10); insufficient contact-dependent immune regulation (e.g., through cytotoxic T-lymphocyte antigen–4), or the conversion of regulatory T cells (Tregs) into Th2 effector cells may aggravate the onset and persistence of type 2 inflammation [37].

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Airway remodeling and various Th cell-driven inflammatory responses, such as Th1 cells, Th2, T follicular helper (T_{FH}), and Th17 are involved in asthma. Type 2 inflammation is mostly caused by the activation of Th2 pathways, which results in elevated levels of IL-4, IL-5, and IL-13 cytokines. In particular, T_{FH} cells (CXCR5CD4T) control the proliferation of B cells and the conversion of immunoglobulin classes in germinal center structures. These cells are responsible for producing the important cytokines IL-4 and IL-21 that stimulate B-cells and regulate the synthesis of IgE in asthma [38].

Additionally, research has demonstrated that T_{FH} cells are efficiently induced by pulmonary dendritic cells in asthmatic lungs, and these T_{FH} cells produce IL-4 to support the secretion of IgE antibodies. Further evidence points to the requirement of a subpopulation of T_{FH} cells (IL-13-Producing-T_{FH}) for the generation of high-affinity IgE antibodies and subsequent allergen-induced allergic reactions. Human allergic asthma was associated with a higher frequency of circulating T_{FH} cells, which produces more IL-4 and IL-21. As a response to the IL-4 secretion from T_{FH} cells, the memory IgG^+ B cells start to produce IgE [39]. It has been demonstrated that the selective activation of (MHC) class II-restricted CD4+ helper T cells (TH) by challenging asthmatics with synthetic peptides derived from an allergen, causes the typical symptoms of asthma, namely airway constriction and sputum eosinophilia [40]. In animal models, TH cell depletion decreases allergic airway inflammation [41], and asthma patients benefit clinically from suppression of TH cell-derived type 2 cytokines (IL-5, IL-13, and IL-4). These findings provide additional evidence of the key role of TH cells in asthma pathogenesis.

4.3. Allergen-Specific B Cells

The role of B cells in humoral immunity to inhaled allergens is crucial. Controversial findings from earlier research on B-cell functions in diseased settings have highlighted the complex roles that B cells play in asthma. It was later revealed that IL-10-producing B cells, a new subset of B cells, can decrease established airway allergic inflammation by generating IL-10 [42]. Moreover, IL-10 exacerbates allergen-induced smooth muscle hyperresponsiveness and promotes Th2 immunity and eosinophil infiltration in allergic dermatitis. These results showed that B cells can release IL-10 in response to aeroallergens [43]. A very recent study revealed that B cell-produced IL-10 can increase immunological response in lung epithelial cells to house dust mite (HDM) stimulation, thereby leading to asthma sensitization [44].

TH2 cells release a range of interleukins, including IL-4 and IL-13, which stimulate B cells to develop into plasma cells that produce IgE. When an allergen is ingested, it interacts with IgE antibodies, which then bind to the high-affinity IgE receptor on mast cells and basophils. This process causes the cells to degranulate and release mediators, such as histamine, cytokines, and proteases, which can cause allergic reactions and potentially fatal anaphylaxis [45].

5. Trained Immunity Biomarkers

5.1. Mitogen-Activated Protein Kinases (MAPKs) Pathway

Mitogen-activated protein kinases (MAPKs) is an important inflammatory pathway in the pathophysiology of asthma, which is linked to inflammation and remodeling in the airways through the activation of immune cells and structure-resident cells. Three significant subgroups of the MAPK family of signaling enzymes include extracellular regulating kinase, p38, and JNK [46].

A previous study showed that ex vivo stimulated children's peripheral blood mononuclear cells (PBMCs) with farm dust extracts and Lipopolysaccharides exhibit anti-inflammatory properties through the upregulation of anti-inflammatory TNFAIP3 and the downregulation of pro-inflammatory NF-κB pathway genes. The pro-inflammatory MAPK pathway is closely linked to the development of adult asthma. When central MAPKs are phosphorylated, kinase cascades build up and activate transcription factors that cause inflammation [47]. It was also demonstrated that asthmatic children express higher p38 levels in naïve T cells [48].

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The most researched MAPK in asthma is P38 MAPK. p38 MAPK exists in four isoforms: p38α, p38ß, p38γ, and p38δ. α and β isoforms appear to be more relevant to asthmatic inflammation, since their levels in human lung cDNA libraries are higher than those of γ and δ isoforms and are linked to the activation of more downstream pathways [49]. In another study, it was demonstrated that p38δ plays a critical role in neutrophil adhesion, cell motility, and chemotaxis which are all necessary for neutrophil extravasation from blood arteries to inflammatory sites [50].

5.2. IL-1 and IL33 Pathway

The genetic regulation of interleukin-33 (IL-33) and its receptor IL-1RL1 in the development of asthma and the molecular mechanisms of this pathway have been extensively researched over the years. IL-33, which is a member of the IL-1 family of cytokines, is essential for several inflammatory processes, including allergic airway disease and type 2 immunological responses. IL-33 was first discovered to be a nuclear factor in human endothelial cells found in high endothelial venules. IL-33 functions as an alarmin in response to tissue damage, necrosis, and pathogenic pathogens, and fibroblasts, endothelial cells, and epithelial cells are the main secretors of IL-33. Upon release, IL-33 binds to a heterodimeric receptor complex made up of IL-1RL1-b (or ST2L) and IL-1RAcP, which causes downstream signaling events that activate immune cells as well as structural cells like fibroblasts, airway smooth muscle cells (ASMCs), and bronchial epithelial cells [51].

Remarkably, increased expression levels of caspase-1, IL-1β, and nucleotide-binding oligomerization domain-like receptors (NOD-like receptor) (NLR) family pyrin domain containing 3 (NLRP3) were linked to neutrophilic asthma. It was shown that targeted therapy directed towards NLRP3 and caspase-1 in mouse models resulted in a reduction of IL-1β production and the suppression of steroid-resistant neutrophilic inflammation and airway hyperresponsiveness [52].

5.3. CD 14

An essential part of the innate immune system is the myelomonocytic differentiation antigen CD14, which is mostly expressed on monocytes and distinct tissue macrophages and less expressed on granulocytes and B-lymphocytes. It functions as a multifunctional receptor for lipopolysaccharides (LPS), endotoxins, and other components of bacterial walls and is present in both membrane-bound form (mCD14) and soluble form (sCD14). LPS binding to sCD14 has been linked to the pathophysiology of bronchial asthma by activating macrophages and bronchial epithelial cells and producing proinflammatory cytokines such IL-1β, TNF-α, IL-6, and IL-8 [53].

Asthma exacerbations have been shown to activate monocytes and macrophages in the bronchoalveolar region and peripheral blood. Monocytes can develop into dendritic cells and macrophages, which in turn control the Th2-mediated immune response and aid in the etiology of asthma. The soluble form (sCD14) and a membrane-bound form (mCD14) of CD14 both positively affect the ratio of Th1 to Th2 cytokines [54].

A previously published study showed that elevated CD14 level in cord blood was a predictor biomarker of wheezing and cough in young children. It is suggested that plasma sCD14 levels are elevated in both adults and children during acute asthma attacks [55].

6. Conclusions

Asthma is a major noncommunicable disease (NCD), affecting both children and adults and is the most common chronic disease among children. Misdiagnosis and underreporting of asthma lead to severity and exacerbation of this disease. The diagnosis and early assessment based on both clinical manifestation as well as biomarker analyses of the serum and sputum may lead to better management of asthma among children.

Creating a panel of reliable and feasible biomarkers at genetic, inflammatory, and immunological levels may result in early and accurate diagnosis, enable the development of new treatments that target specific pathways, and suggest the possibility of personalized treatment plans that are more likely to be effective based on the patient's specific inflammatory and immune response

profile. The advancements in the understanding of asthma biomarkers represent a significant step forward in the management of asthma in children. They not only offer the potential for early detection but also pave the way for more targeted and effective treatments, ultimately improving outcomes and quality of life for young asthma patients.

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