



Risk Factors and Mechanisms Leading to Preschool Recurrent Wheeze and Asthma

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Abstract

Preschool recurrent wheezing is a prevalent and heterogeneous condition that can develop into childhood asthma, significantly damaging public health. Preschool recurrent wheeze and asthma

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are influenced by a multifactorial interplay of biological, environmental, early-life, behavioural, and psychosocial factors. Genes as *GSDMB*, *ORMDL3*, *CDHR3*, *ANXA1*, and *IL33/IL1RL1*, and methylation of cell-type-specific CpG-sites, are associated with airway-remodelling, increased inflammatory responses, and enhanced susceptibility to environmental factors. Biomarkers, such as allergen sensitization, blood eosinophil levels, fractional exhaled nitric oxide, and volatile organic compounds, may guide treatment decisions based on the type of immune response in wheezing episodes. Several asthma-predicted clinical indices have been developed, and some of them have been validated. Although lower airway samples obtained through bronchial-alveolar lavage and biopsy in young children are limited, they are essential in understanding the pathophysiology and developing personalized treatment of recurrent preschool wheezing. Early evaluations of lung function, airway hyperresponsiveness, and bronchodilator response can be valuable objective tools. However, due to physiological variability and inconsistent methods and definitions, these tests cannot confirm or rule out a diagnosis of asthma at preschool age. Future research should investigate the interplay of factors across biological, environmental, and social domains to enhance predictive models and inform targeted interventions that promote health equity and reduce the global burden of preschool recurrent wheeze and asthma.

Keywords

preschool; recurrent wheezing; asthma; risk factors; mechanisms; genetic; epigenetic; lung function

1. Introduction

Recurrent preschool wheezing, defined as more than one confirmed wheezing episode before the age of 6 years (1), is a common and heterogeneous condition that can progress into childhood-onset asthma and poses a tremendous burden on public health. Sometimes, recurrent preschool wheezing is also called preschool asthma. However, not all preschool children with wheezing will have an asthma diagnosis by the time they reach school age. It is challenging to accurately predict which preschool children with recurrent wheezing will develop asthma. Several predicted clinical indices have been developed, and some of them have been validated. Furthermore, definitions of wheezing and asthma in preschool children vary among studies, which hampers uniformity. It is essential to understand the various factors associated with recurrent wheezing in preschool children, as this helps elucidate the mechanisms contributing to the development of asthma later on. This can help us identify children with recurrent wheeze that will progress into childhood-onset asthma early on, providing opportunities for early intervention. This review presents the most recent findings on risk factors for recurrent preschool wheezing and the underlying role of genetics and epigenetics in the progression from wheeze to asthma. Additionally, various methods for identifying the mechanisms of different wheezing phenotypes will be discussed, including non-invasive and invasive approaches. Finally, the role of lung function testing in diagnosing asthma early on at preschool age will be explored.

2. Factors associated

Recurrent wheeze in preschool children is a critical public health challenge due to its high prevalence and significant impact on children's quality of life and healthcare systems.(1) We summarize the factors associated with recurrent wheezing and asthma in preschool children, organized into six major categories: (a) biological, genetic and familial factors; (b) environmental and socio-economic factors; (c) early-life exposures; (d) behavioral and social factors; (e) past medical conditions; and (f) parental and prenatal stress exposure (Figure 1).

Biological, genetic, and familial factors

The main reported factors in the literature include a history of (first-degree) family with atopy, asthma, allergic rhinitis, and/or a history of wheezing. (2–18) Furthermore, a child has a higher risk of developing recurrent preschool wheezing and subsequent childhood-onset asthma if it is a boy, (7, 11, 14, 16, 19–23) and if atopy with elevated IgE antibody levels, eosinophilia and/or eczema is present in the child, some of which are used in the Asthma Predictive Index (API). (6, 7, 14, 15, 17, 24–26) Specific sensitization to, for instance, food, aeroallergens, mite, grass, cat, and dog allergens has been reported as a risk factor as well. (3, 4, 27, 28). Additionally, fractional exhaled nitric oxide (FENO) 30 ppb (29) and early low lung function are also associated with recurrent preschool wheezing. (30, 31)

Environmental and socio-economic factors

Numerous environmental factors have been associated with recurrent preschool wheezing. Tobacco smoke exposure, maternal smoking during pregnancy, and exposure to traffic-related air pollutants or usage of gas as cooking fuel are known risk factors. (6, 7, 16, 21, 22, 24, 31–39) Although some studies suggest exposure to furry pets and farm dust in the first years of life mitigates the risk of asthma and allergy (40, 41), other studies have found a positive association between pet keeping/exposure during the first year of life and recurrent preschool wheezing. (10, 14, 16, 42, 43) Poor housing quality, in combination with home dampness, molds, and unsatisfactory ventilation in the sleeping area, has also been associated. (2, 11, 16, 18, 32, 44, 45) Low socioeconomic status, associated with low per capita income, and low parental post-primary education are other risk factors for recurrent preschool wheeze. (7, 22, 45)

Early-life exposures

Exposures associated with recurrent preschool wheezing start in the womb. Preeclampsia and higher gestational weight gain, along with low intake of fish oil components eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), vitamin D, calcium, dairy products, and/or vegetables have been linked, as well as low or high consumption of meat and high consumption of pasta during pregnancy. (4, 6, 11, 19, 46–50) If a child is born via cesarean, prematurely, with a low birth weight, in warmer climates, or during the second half of the year, it also has a higher risk of developing recurrent preschool wheeze. (6, 7, 14, 16, 34, 39, 40, 45) Exclusive breastfeeding, on the other hand, seems to be protective (2, 15, 18, 51–54), while antibiotics taken by the child increased the risk of recurrent preschool wheezing and asthma. (10)

The strength of the association between early-life exposures and asthma risk varies depending on the specific exposure and the cohort studied. For instance, while some observational studies and randomized trials suggest that low prenatal or early postnatal vitamin D levels may increase the risk of asthma, other studies have found no significant associations (55), and meta-analyses have yielded inconclusive results. Similarly, associations between asthma and dietary factors, such as high consumption of pasta or ultra-processed foods, have been observed in specific populations but not universally replicated. Therefore, these associations should be interpreted with caution. Further prospective studies in diverse populations are needed to determine the consistency and causality of these findings.

Behavioral and social factors

Having older siblings, attending daycare, and playing outside have been associated with recurrent preschool wheezing. (6, 14, 15, 17, 19, 20, 45, 56) Certain dietary intake, such as low fruit consumption, high fast-food consumption, and high intake of polyunsaturated fats, has also been associated. (15, 50) Other risk factors for recurrent preschool wheeze include lack of social support, single parenting, excess weight in children, frequent use of paracetamol, and bed-sharing in toddlerhood. (21, 36, 44, 57–59)

Past medical conditions

Respiratory tract infections (including severe bronchiolitis and pneumonia) are associated with increased risk of recurrent preschool wheezing. (2, 10, 60) This was specifically observed with respiratory syncytial virus, rhinovirus, or mycoplasma infection, particularly in children who had a severe infection in the first 2 years of life and/or required hospital admissions. (4, 8, 12, 15, 25, 38, 61–64) For example, children hospitalized with rhinovirus-induced wheezing during infancy are up to ten times more likely to develop asthma by school age. RSV has also been associated with a two- to fourfold increase in asthma risk, particularly when a severe lower respiratory tract infection occurs during the first year of life. (65) The proposed mechanisms include virus-induced epithelial damage, immune dysregulation favoring Th2 responses, and altered airway development during a critical period of lung growth. (66) Furthermore, an association was found between the presence of food allergy, allergic rhinitis, and the emergence of recurrent wheeze and asthma. (2, 3)

Parental and prenatal stress exposure

Findings from birth cohort studies suggest mild to moderate effects of pre- or post-natal stressors (e.g., depression, violence) or chronic stress on wheezing and worse asthma-related outcomes in preschool children. (67–70) Most studies have focused on mothers, but one study of 339 twin pairs showed that paternal post-traumatic stress disorder and paternal or maternal depression in the children's first year of life were associated with worse asthma symptoms at age 1 year, with parental depression also linked to hospitalizations at age 3 years due to asthma symptoms. (71) More recently, a study of 2,056 mother-child dyads found that maternal stressful life events during pregnancy were associated with wheezing in the last 12 months between the ages of 4 and 6 years, but this association was only significant in boys. (72) In another recent study of predominantly Black and Latinx mother-child pairs, the estimated effects of maternal prenatal stress were modified by BMI, as

prenatal stress was only associated with asthma symptoms in children ages 1–5 years born to mothers who were obese during pregnancy. (73)

3. Role of Genetics and Epigenetics

The strongest risk factor to predict whether a child will develop asthma is a first-degree relative with asthma. (74) This suggests that shared (epi-)genetic and/or environmental factors play a crucial role in the development of asthma. Twin studies showed that monozygotic twins have up to four times the concordance rates for developing asthma compared to dizygotic twins. (75, 76) Furthermore, the correlation between ages at onset of asthma was most strongly in monozygotic twins, compared to dizygotic twins (0.37 vs 0.09). (75)

Two approaches to investigating the genetics of childhood-onset asthma are Genome-Wide Association Studies (GWAS) and Candidate Gene Studies.(77) Combining GWAS (to identify potential genes) and candidate gene studies (to verify potential genes and their interactions in more characterized participants) can lead to a better understanding of the development of recurrent preschool wheeze and asthma in children.

Childhood-onset asthma was more than twice as heritable as adult-onset asthma (25.6% vs 10.6%) in a large GWAS from the UK Biobank. (78) As a consequence, 123 identified single-nucleotide polymorphisms (SNPs) were associated with childhood-onset asthma, compared to only 56 SNPs in adult-onset asthma, of which 37 overlapped. Additionally, the effect size of genetic associations in childhood-onset asthma was more than three times larger than in adult-onset asthma (79), confirming that the earlier asthma develops, the more genetic it tends to be.

The most replicated locus strongly associated with recurrent preschool wheeze and childhood-onset asthma is the 17q12–21 locus, also known as ‘17q’.(77) Well-known genes associated with the 17q locus are orosomucoid 1-like 3 (*ORMDL3*) and gasdermin B (*GSDMB*). (77) SNPs in both genes have been associated with childhood-onset asthma in children of different ancestries. (80) *ORMDL3* and *GSDMB* are implicated in increasing airway hyperreactivity and remodelling. (77) Gene-environment studies of this locus have shown that certain exposures can reduce or exacerbate the likelihood of wheeze or asthma in children with the 17q risk alleles. Early life exposure to furry pets could attenuate the increased risk of wheeze, while early life exposure to tobacco smoke or viral infections could enhance the risk of developing asthma. (81)

Although 17q has been associated with childhood-onset asthma in general, other genes have been linked to specific phenotypes. For example, gene variants in Cadherin-related family member 3 (*CDHR3*), located on chromosome 7, were associated with hospitalizations for severe asthma exacerbations in children aged 2–6 years. (82) *CDHR3* is expressed as a transmembrane protein in ciliated airway epithelial cells and encodes the Rhinovirus C receptor. Interestingly, children with a carry-risk genotype of *CDHR3* are more susceptible to Rhinovirus C infections. (82) Moreover, lower expression of Annexin A1 (*ANXA1*), a gene located on Chr9q21 and encoding an anti-inflammatory protein, has been specifically

associated with early onset and persistent wheeze. (83) Furthermore, interleukin-33 (*IL33*) and its receptor interleukin-1 receptor-like 1 (*IL1RL1*) have been associated with different preschool wheeze patterns, including persistent wheeze; these genes play an important role in activation of type 2 inflammation. (77) Table 1a presents a selection of genes associated with recurrent preschool wheeze and their proposed function.

Genetic factors cannot fully explain the heritability of childhood-onset asthma. Therefore, epigenetics, meaning ‘above genetics,’ has gained significant interest. Epigenetics focuses on heritable characteristics that alter gene expression without changing the DNA sequence. Environmental factors, such as maternal smoking during pregnancy or exposure to air pollution after birth, may influence epigenetic changes. (84) The epigenome is cell-type specific and strongly depends on age. (85) DNA methylation is the most studied epigenetic mechanism in childhood-onset asthma. By detaching or attaching a methyl group to a Cytosine, situated next to a Guanine (CpG), gene transcription can be increased or reduced, respectively. (86)

DNA methylation is often studied in blood or nasal epithelial samples after preschool age. (87–90) A meta-analysis of childhood asthma examined DNA-methylation data from whole blood in children aged 4–5 and 8 years. (91) Fourteen replicable hypomethylated CpG-sites were associated with childhood-onset asthma from age four onwards; these CpG-sites were annotated as associated with activated eosinophils. Five of these 14 CpG sites were also replicated in respiratory epithelial cells of 16-year-old children with asthma. However, (hypo)methylation of these CpGs in cord blood did not significantly predict asthma development at preschool age. In contrast, another study found a CpG-site within hexokinase-1 (*HK1*) in whole blood to be associated with adolescent asthma, while higher expression of *HK1* in cord blood was predictive of preschool wheeze, indicating a possible relationship between *HK1* and the onset of preschool asthma. (92) Moreover, in a large meta-analysis of the association between cord blood DNA and subsequent childhood asthma, six CpG-sites in cord blood were found to predict childhood asthma; however, the cross-sectional associations of blood DNA-methylation with school-aged asthma were much stronger. (93) This phenomenon, where the associations of DNA-methylation are stronger after the onset of disease, is also observed in other traits (94) and suggests that most of the DNA-methylation changes in blood appear to be the result, not the cause, of asthma. Table 1b presents a selection of CpG sites associated with recurrent preschool wheeze and their proposed functions.

4. Non-invasive methods for identifying the mechanisms of wheezing phenotypes

Birth cohort studies have categorized phenotypes of preschool wheezing based on their timing and severity, with the most common types being early transient wheeze, late-onset wheeze, and persistent wheeze. (95, 96) However, endotypes describe these distinct pathophysiological mechanisms at a cellular and molecular level. This is important because, despite having similar clinical symptoms, children may respond very differently to the same therapeutic interventions. (97) Biomarkers can be utilized to identify endotypes of

preschool wheeze and may have a greater impact when used to predict response to targeted therapies. (98) Biomarkers commonly used to assess preschool wheeze include allergen sensitization, blood eosinophil levels, fractional exhaled nitric oxide (FeNO), and volatile organic compounds (VOCs), which can help identify the presence of airway inflammation and guide treatment decisions based on the type of immune response involved in the child's wheezing episodes. (99)

Sensitization is one of the main risk factors for developing childhood-onset asthma (100) as evidenced by its inclusion as one of the six predictive factors in the Pediatric Asthma Risk Score (PARS). (101) A study of 7,719 children participating in one of five birth cohort studies identified four trajectories of wheeze across the ages of 0.5–18 years: early transient, intermittent, late-onset, and persistent. (102) All wheezing phenotypes were associated with sensitization at an early school age, with a higher risk for those with late-onset or persistent wheeze. (102) Comparing temporal patterns, children with persistent and intermittent wheeze were more likely to have wheeze preceded by sensitization, whereas sensitization preceded wheeze in late-onset wheezers. (102) Approximately 40% of recurrent preschool wheezers are sensitized, making them more likely to be steroid-responsive. In a trial of asthmatic children aged 12–59 months, aeroallergen sensitization was the strongest predictor of those who had the best response to daily inhaled corticosteroids (ICS), with this response further increased in those with blood eosinophils $\geq 300/\mu\text{L}$. (103) This suggests that phenotyping with sensitization and blood eosinophils may be useful in guiding treatment selection. (103)

Due to its key role in asthma risk, severity, and persistence, sensitization was one of the primary inclusion criteria for preschoolers enrolled in the Preventing Asthma in high-Risk Kids (PARK) trial. (104) Since the cascade of biological responses to allergens is primarily mediated through IgE antibodies, PARK researchers hypothesized that early blockade of IgE and IgE mediated responses with anti-IgE monoclonal antibody treatment (omalizumab) would prevent the development and reduce the severity of asthma in children aged 2–3 at high risk for developing asthma. (104) Although the results will not be available until 2027, PARK will provide significant insights into the pathobiology of asthma and potentially other IgE-mediated allergic diseases, such as food allergy and allergic rhinitis, moving science closer to asthma prevention. (104)

Blood eosinophil counts also correlate with the degree of airway inflammation. Eosinophils are recruited to the lungs after exposure of the airways to an antigen, which stimulates the release of Th2 cytokine-producing T-cells, as well as IL-5 and eotaxin. (105) In preschool children, systemic eosinophils are elevated in those who wheeze and are associated with the persistence of asthma by school age. (106) In a study of 219 infants until 18 months of age with recurrent wheeze, a blood eosinophilia count $\geq 470/\text{mm}^3$ was the most important risk factor for wheeze that persisted at age six. (107) The authors suggest that a lack of eosinophilia in wheezy infants without ongoing infection could predict future remission of wheeze. Recently, data from three trials involving 1074 preschool children with recurrent wheeze showed that high eosinophil counts at multiple cut points, ranging from ≥ 150 cells/mL to ≥ 350 cells/mL, all indicated an increased risk of exacerbation and a higher occurrence of hospitalization. (108) These results suggest that blood eosinophil cut points

may be helpful in clinical assessment and future studies of exacerbation and treatment response in preschool children with recurrent wheeze.

It is essential to note that both biomarkers—elevated blood eosinophils and allergic sensitization—are components of previous clinical scores, such as the API (109) and the modified API (mAPI). (110) It is well known that clinical scores have different sensitivities, specificities, positive and negative predictive values, and likelihood ratios (LR). In terms of maximizing the ability to diagnose a disease, the likelihood ratio (LR) may be the best parameter to follow, as it reflects the magnitude by which the pretest probability increases (or decreases) after applying the score, thereby helping the physician rule out, confirm, or continue investigating a diagnosis with new tests. The original API had a +LR of 7.4 and a -LR of 0.75.

As an example, if physicians from countries with high (i.e., Brazil), medium (i.e., Chile), and low (i.e., China) asthma prevalence use the original stringent API to identify the risk of asthma in their patients, the probability for asthma increases by 2, 4, and 7 times, respectively (because the pretest to posttest probability of asthma moves from 40 to 80%, 14 to 62%, and 3 to 21%, respectively). That means an acceptable +LR, but since the -LR is not good enough, it cannot be used to “rule out” the probability of developing asthma. The other validated scores have lower +LR and -LR (e.g., 2.5 and 0.53 for PIAMA; 2.5 and 0.4 for APT; and 3.25 and 0.41 for PARS), with a modest +LR and low -LR, some of these tools may be useful to “rule out” asthma but less helpful to diagnose it. (111) Recently, a longitudinal birth cohort (n=339) suggested, for the first time, that original API could be used as a diagnostic tool, not only as a prognostic tool, in toddlers and preschoolers; in this population, the +LR was 6.4, and the overall accuracy was ~84%. (112)

Another measure of eosinophilic airway inflammation is FeNO, which correlates well with airway hyperresponsiveness and atopy in children. (113) Exhaled breath FeNO plays a role in vasodilation and bronchodilation, and its production increases in acute and chronic inflammation. (114) In the Prevention and Incidence of Asthma and Mite Allergy (PIAMA) study, high FeNO at age four was associated with intermediate onset and persistent wheeze at age eight compared to those with transient early wheeze or never wheezed. (115) PIAMA also showed that higher FeNO levels at 4 years were associated with a physician diagnosis of asthma at 7 years and a higher prevalence of wheezing between 5 and 8 years of age. (116) Other studies have also shown FeNO to be higher in children with recurrent wheeze (117) and children with persistent wheeze than those with transient wheeze. (113) As a biomarker of Th2 inflammation, FeNO may have utility in treatment decision-making and as a measure of treatment adherence. (118) The European Respiratory Society task force recommends using FeNO levels of ≥ 25 ppb in children aged 5 years and above as a cutoff for those with symptoms consistent with asthma, (119) but more evidence is needed to establish the utility and feasibility of FeNO in preschool children to identify type 2 inflammation. (118) However, children often find the procedure demanding to perform, especially at preschool age, reducing the likelihood of a successful test. (120) Additionally, factors such as rhinovirus infection and the intake of nitrite-containing foods can alter FeNO measurements. (121)

Another component of exhaled breath is VOCs, which have gained considerable interest as a biomarker for asthma. (122) VOC levels are significantly different in asthma and are related to disease activity and phenotypes. (123) Smolinska *et al.* evaluated VOCs in children aged 2–6 years for predicting which recurrent preschool wheezing children will develop asthma at school age (124) and found a set of 17 VOCs that could discriminate between transient recurrent wheezing and wheezing that proceeds into asthma with a prediction rate of 80%. (124) This suggests that VOCs can predict subsequent asthma development and may help inform the start of early treatment. As a diagnostic tool, VOCs have been shown to distinguish asthmatics from healthy children. The exhaled breath of 63 asthmatic children and 57 healthy controls, aged 5–16, was analyzed for VOCs. (125) Eight components distinguished asthmatic children from healthy children with 92% accuracy, 89% sensitivity, and 95% specificity, highlighting that a limited number of VOCs can effectively identify children with asthma. (125) Further replication of VOCs results to predict asthma at independent laboratories is eagerly awaited. Like FeNO testing, measuring VOCs can be challenging for younger children to perform accurately, and the results may be influenced by factors such as diet and environmental exposures. (126) Table 2 summarizes non-invasive biomarkers of preschool wheeze.

5. Invasive methods for identifying the mechanisms of wheezing phenotypes

As emphasized above, recurrent preschool wheezing is heterogeneous in the time course of symptom onset and persistence (curricular heterogeneity), and in the pathological mechanisms underlying the clinical presentation (etiological heterogeneity). (1, 127) The etiological heterogeneity is also reflected in differences in responses to prescribed treatments. (103, 118) For example, only 30–40% of preschool children with recurrent wheeze who require treatment show a notable therapeutic response to maintenance ICS, and these patients are often characterized by the presence of aeroallergen sensitization and/or blood eosinophilia. (103) However, despite this heterogeneity in long-term outcomes, disease mechanisms, and treatment responses, the clinical presentation at the time of contact with healthcare professionals is similar for the majority of patients. (127)

The differences in temporal patterns (with wheezing either remitting, relapsing, or persisting) have been a topic of major interest for almost four decades. (128) While this curricular heterogeneity may reflect distinct mechanisms to a certain degree, (83) it is essential to emphasize that very similar temporal or clinical patterns of wheezing (“wheeze phenotypes”) may arise through different pathophysiological mechanisms. (129, 130) This should not be surprising, given that multiple, often very different gene-environment combinations can produce very similar phenotypic clusters. (131) Consequently, symptom-based diagnostic labels, such as episodic viral wheeze, multiple-trigger wheeze, and transient or persistent wheeze, are of limited value in clinical practice. (1, 96)

Since clinical presentation may be a relatively poor indicator of underlying pathophysiology, the end-organ pathology may be crucial for ascertaining disease mechanisms. In adult asthma, invasive studies using bronchoscopy have been crucial for better understanding the

molecular and cellular mechanisms, the role of lower airway inflammation and infection, and have contributed to developing novel treatment strategies. (132) However, there are major ethical, technical, and scientific challenges that need to be overcome to enable the collection of lung tissue from young children and help our understanding of the mechanisms underpinning the onset, severity, and progression of early childhood wheezing/asthma. (133) This includes, but is not limited to, the fact that invasive procedures for research purposes are not ethically acceptable in this age group. (133) Given the ethical considerations, one approach to collecting precious lower airway samples is to utilize data from young patients undergoing clinically indicated bronchoscopy, typically due to severe symptoms. A limited number of such studies have produced invaluable results, highlighting the differences between early childhood severe wheezing and asthma in school-age and adulthood. Inference from these studies suggests that lower airway pathology in children with severe wheeze is age-dependent. (134, 135) For example, pioneering early studies conducted two decades ago showed no evidence of submucosal airway inflammation and/or airway remodeling in infants and children under 2 years, even in those who were atopic. (134) In contrast, the pathologic features characterizing adult asthma, including submucosal eosinophilia and reticular basement membrane (RBM) thickening, were apparent by approximately 3 years of age, while the clinical presentation remained almost identical across the age ranges. (135) Similarly, another study among young preschool wheezers showed no evidence of bronchoalveolar lavage (BAL) T2 inflammation, but described increased BAL CD8+ lymphocytes and neutrophils, as well as endobronchial RBM thickness. (136) A study at a similar age (approximately 1 year) reported increased total BAL leukocytes (but not eosinophils) compared to healthy controls, suggesting that airway inflammation, when present in infants with wheeze, differs from the patterns observed in adult asthma. (137) This is consistent with the analysis of BAL from young children with wheeze, which revealed predominantly neutrophilic airway inflammation. (138) In contrast, a study in children with multiple trigger wheeze aged between 2 and 10 years described airway pathology typical of adult asthma (eosinophilia and thickened RBM), even amongst non-atopic children. (139)

A hypothesis-generating data-driven analysis of lower airway inflammation and infection from BAL of children with severe preschool wheeze revealed four pathological clusters characterized by different patterns of allergic sensitization, blood eosinophils, and BAL microbial infection and inflammation. (140) Notably, most patients in this study were neither sensitized nor eosinophilic. Other studies have described a subgroup of preschool children with recurrent wheeze and predominant lower airway neutrophilia, whose disease is corticosteroid-refractory. (141) However, many children with airway neutrophilia have bacterial infection (140), and it remains unclear whether airway neutrophilia reflects a protective response to control infection or whether neutrophils are causing harm.

Taken together, the above studies suggest that amongst young children with severe wheeze, airway eosinophilic inflammation and remodeling are rarely present in infancy, but develop between infancy and preschool age, reaching adult patterns by school age in those who progress to severe asthma. Thus, persistence of symptoms does not reflect the persistence of the same pathophysiological mechanism. Follow-up studies have suggested that airway smooth muscle (but not inflammation) in preschool airway biopsies was predictive of

school-age asthma, indicating that airway remodeling, rather than inflammation, should be a central target for disease modification. (142)

Given the scarcity and extraordinary value of lower airway samples from young children in understanding the pathophysiology of early childhood wheezing and its progression, quality datasets from multiple studies should be combined in a major international collaborative effort to enable analyses that would otherwise be impossible in any single study.

6. Role of lung function

The 2018 Lancet Commission on Asthma (143) emphasizes measuring lung function in children. Such objective assessments could enhance our ability to detect abnormalities early and facilitate the diagnosis of asthma. However, it is important to note that most preschool children with asthma have normal lung function, regardless of whether it is measured by spirometry, impulse oscillometry (IOS), or the forced oscillation technique (FOT). Moreover, while IOS and FOT are performed during tidal breathing and are therefore easier to perform in preschoolers than spirometry, they also exhibit greater variability. Another barrier is the lack of clear definitions of airway hyperreactivity (AHR) or bronchodilator response (BDR) in this age group (144), which would be necessary to support the early diagnosis of asthma.

A recent study of 851 preschoolers with asthma (145) showed that 86% had normal spirometry while 40% had borderline AHR to methacholine (defined as $4 \text{ g/L} < \text{PC}_{20} < 16 \text{ g/L}$). Oostveen et al. (146) reported that only 13% to 23% of 313 preschool children with persistent wheezing had BDR measured by FOT and that the prevalence depended on the definition of BDR ($R_4 > 5.5$; $AX > 31.0$, or $AX/AX > 81\%$). Similarly, Shee and colleagues (147) reported that only 18% of 592 young school-aged children with asthma had BDR measured by spirometry ($FEV_1 < 12\%$), 9% when BDR was defined by IOS ($R_5 < 34\%$ or $X_5 < 50\%$), and that only 8% had BDR with both definitions. More recently, Grell et al. (148) evaluated preschool children with persistent asthma and found that 23% had spirometry-defined BDR ($FEV_1 < 10\%$ and/or $FVC < 10\%$) and only 12% had IOS-defined BDR ($R_5 < 40\%$, $X_5 < 50\%$, or $AX < 80\%$). Meoli et al. (149) reported similar findings in preschool children with suspected asthma: 19.4% exhibited BDR by spirometry ($FEV_1 < 12\%$) versus 11.1% with BDR by IOS ($R_5 < 40\%$, $X_5 < 50\%$, or $AX < 80\%$).

Lezana and colleagues (150) found no differences in basal lung function and BDR by IOS or spirometry between 108 recurrent wheezing preschoolers with a positive and negative API. However, those preschoolers with positive API without ICS had significantly lower basal R_{20} and lower BDR ($\% \text{ FEF}_{25-75}$ and $FEV_{0.5}$) than those with positive API and ICS. Later, Arikoglu et al. (151) reported, in 115 preschoolers with recurrent wheezing, higher basal R_5 - $R_{20}\%$ predicted levels by IOS in those with a positive mAPI compared to those with a negative mAPI. In the ROC analysis, R_5 - $R_{20}\%$ levels >14.4 had a sensitivity of 75%, specificity of 53%, and $\text{AUC} = 0.66$ for predicting a positive mAPI.

Others have evaluated the capacity to predict asthma at school age using preschool lung function in a high-risk birth cohort and recurrent preschool wheezers. Knihtila (152)

reported that spirometry at age five would yield a fair +LR of asthma by age 8 (+LR 2.37 using FEV₁ and 2.2 with FEV₁/FVC), but IOS at age 4 yielded a more modest +LR (1.85 with R5-R20 and 1.84 with AX). Grell (148) reported that AX, R5-R20, and R5 had the best +LR for the probability of abnormal spirometry during school age (+LR= 50, 10, and 7.1, respectively), while R20, R5, and AX were the best IOS parameters for discriminating BDR in schoolchildren (+LR = 3.4, 2.9, and 2.8, respectively).

While early assessment of lung function, AHR, and BDR are helpful objective tools when there is clinical suspicion, significant physiologic variability, and inconsistent methods and definitions exist, they cannot confirm or completely exclude a diagnosis of asthma. This highlights the need for more –and larger– studies to standardize methods for each lung function test, determine distribution by age or other characteristics (i.e., influence of ICS use), determine the corresponding z-scores, explore the utility of combining different lung function tests, and define factors that will aid in disease diagnosis (e.g., accuracy, positive and negative LRs, and minimally important clinical difference [MCID]).

CONCLUSIONS

There are multiple risk factors associated with preschool asthma and recurrent wheeze, including but not limited to the male gender, a positive family history for asthma, and exposure to smoke, either prenatal or in the first years of life. Genes as *GSDMB*, *ORMDL3*, *CDHR3*, *ANXA1*, and *IL33/IL1RL1*, and cell-type specific (hypo)methylated CpGs relate to airway remodelling, increased inflammatory response, and amplified susceptibility to viruses and other environmental factors. Risk factors as sensitization, eosinophilia, eczema, high FeNO levels, and VOCs in exhaled breath, combined with (epi)genetic risk variants, may be used as biomarkers to guide treatment options between different wheezing phenotypes and endotypes in the future. Lower airway samples from preschoolers are essential to understanding the underlying pathophysiology. They suggest that airway eosinophilic inflammation and remodeling are rarely present in infancy, but develop between infancy and preschool age. Several predicted clinical indices for asthma have been developed, and some of them have been validated. Although early assessment of lung function can be helpful in diagnosing preschool asthma, it cannot confirm or completely exclude asthma due to physiological variability and inconsistent methods and definitions. Future research should investigate the interplay of factors across biological, environmental, and social domains to enhance predictive models and inform targeted interventions that promote health equity and reduce the global burden of recurrent preschool wheezing.

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Abbreviations:

AHR	Airway hyperreactivity
ANXA1	annexin A1

API	Asthma Predictive Index
BAL	bronchoalveolar lavage
BDR	bronchodilator response
CDHR3	cadherin-related family member 3
CpG	Cytosine situated next to a Guanine
DHA	docosahexaenoic acid
EPA	eicosapentaenoic acid
FOT	forced oscillation technique
FENO	fractional exhaled nitric oxide
GSDMB	gasdermin B
GWAS	Genome-Wide Association Studies
IOS	impulse oscillometry
ICS	inhaled corticosteroids
IL1RL1	interleukin-1 receptor-like 1
IL33	interleukin-33
LR	likelihood ratio
ORMDL3	orosomucoid 1-like 3
MCID	minimally important clinical difference
PARS	Pediatric Asthma Risk Score
PIAMA	Prevention and Incidence of Asthma and Mite Allergy
PARK	Preventing Asthma in high-Risk Kids
RBM	reticular basement membrane
SNPs	single-nucleotide polymorphisms
VOCs	volatile organic compounds

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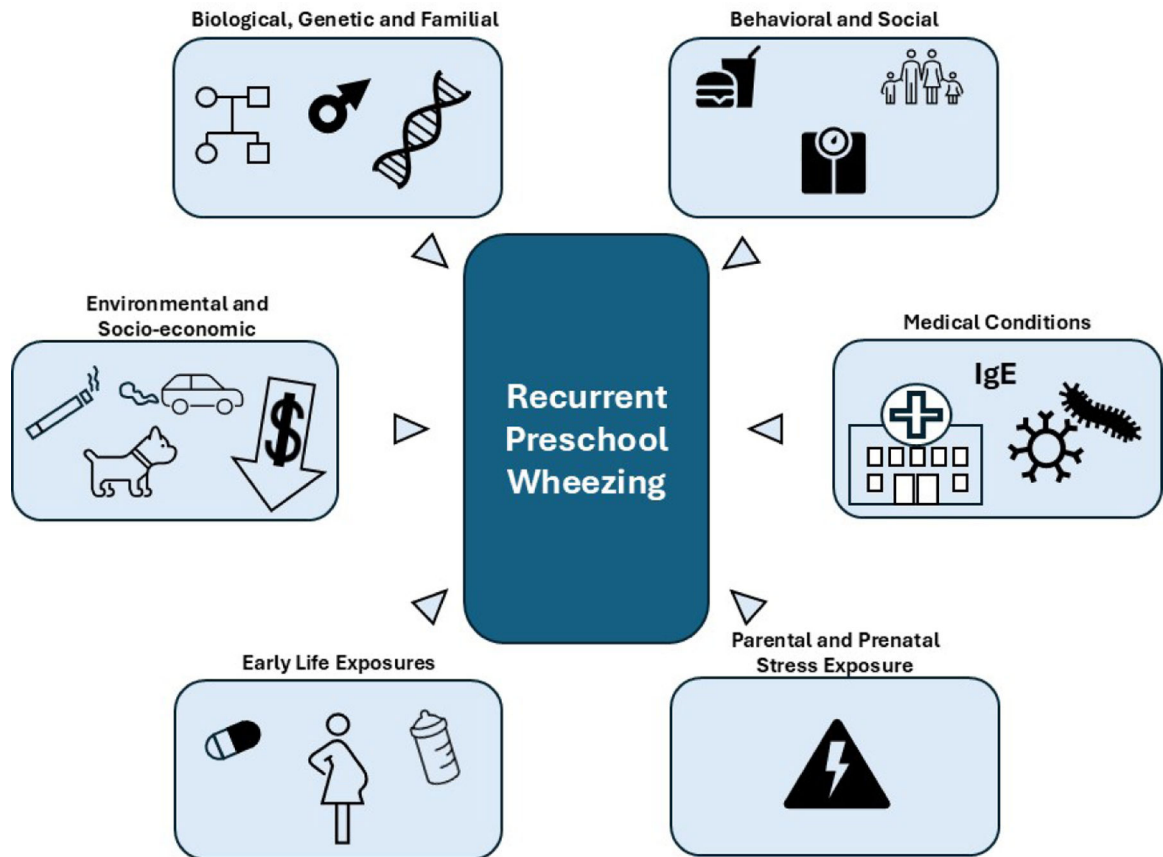


Figure 1. Factors associated with recurrent wheezing and asthma in preschool children.

Table 1a: Selection of genes associated with recurrent preschool wheeze and their proposed function

Gene	Genomic location	Gene expression	Function of the protein	Proposed disease mechanism in preschool wheeze	Associated phenotype
<i>ANXA1</i> (83)	Chr9q21	Protein is located at the membrane, cytoplasm, and is secreted outside the cell	<i>ANXA1</i> binds to phospholipids and plays a crucial role in anti-inflammatory activity.	Loss of <i>ANXA1</i> could increase inflammation and trigger Type 2 inflammation.	Recurrent preschool wheeze
<i>CDHR3</i> (153)	Chr7q22	Protein located at the cell membrane	<i>CDHR3</i> is involved in calcium-dependent cell-cell adhesion and acts as a receptor for human rhinovirus C.	Altered expression and function of the <i>CDHR3</i> -receptor can make the host more susceptible to rhinovirus C infection, which can result in more severe infections and wheezing	Asthma exacerbations
<i>GSDMB</i> (154)	Chr17q21	Precursor of a pore-forming protein in the plasma membrane and cytoplasm	<i>GSDMB</i> is a precursor of the protein that triggers pyroptosis by forming pores in membranes. It also regulates epithelial cell repair.	<i>GSDMB</i> could increase airway responsiveness and remodelling	Recurrent preschool wheeze/childhood-onset asthma
<i>IL33/ILIRL1</i> (155)	Chr9p24/ Chr2q12	Cytokine and the transmembrane and soluble IL-33 receptor	<i>IL33</i> acts as an alarmin and binds to the <i>IL1R1</i> receptor, which can activate an immune response.	<i>IL-33</i> , linked to <i>IL-IRL1</i> , may enhance a type 2 inflammatory response in wheezing children.	Recurrent preschool wheeze/childhood onset asthma/asthma exacerbations
<i>ORMDL3</i> (156)	Chr17q21	Transmembrane protein located in the endoplasmic reticulum	<i>ORMDL3</i> can regulate sphingolipid biosynthesis and plays a role in regulating calcium levels within the endoplasmic reticulum.	<i>ORMDL3</i> could increase airway hyperreactivity and airway remodelling.	Recurrent preschool wheeze /childhood onset asthma

Abbreviations: *ANXA1*: Annexin A1; *CDHR3*: cadherin-related family member 3; *GSDMB*: gasdermin B; *IL33*: Interleukin-33; *IL1R1*: Interleukin-1 receptor-like 1; *ORMDL3*, orosomucoid 1-like 3

Table 1b: Selection of CpG-sites associated with recurrent preschool wheeze and their proposed function

CpG-site	Cell type	Closest gene	Genomic location	Gene codes for	Function of the protein	Proposed disease mechanism in preschool wheeze	Associated phenotype
cg16658191 (92)	Whole blood, cord blood	<i>HK1</i>	Chr10q22	Protein in the outer membrane of mitochondria	HK1 is used in the first steps of glucose metabolism pathways, and HK1 can induce apoptotic resistance	Higher apoptotic resistance in pro-inflammatory cells can prolong inflammation and may increase the risk of preschool wheeze	Recurrent preschool wheeze, childhood-onset asthma (<18 years)
cg01901579 (91)	Whole blood, purified eosinophils, nasal respiratory epithelial cells	<i>DICER1</i> , <i>CLMN</i>	Chr14q32	<i>DICER1</i> codes for a protein that acts like a ribonuclease. <i>CLMN</i> codes for a transmembrane domain protein	<i>DICER1</i> can repress gene expression and is also known as a strong antiviral agent. <i>CLMN</i> is predicted to enable actin filament binding activity.	The <i>DICER1</i> protein can act as a strong antiviral agent against RNA viruses. The role of <i>CLMN</i> is unknown. Eosinophils from asthma patients show lower methylation and transcriptional signatures in whole blood indicate increased activation of the eosinophils, which could be an early marker of wheeze.	Childhood-onset asthma (4/5 years old and 8-year-olds)
cg13628444 (91)	Whole blood, purified eosinophils, nasal respiratory epithelial cells	<i>RAPGEF1</i> , <i>MED27</i>	Chr9q34	<i>RAPGEF1</i> codes for a guanine nucleotide-releasing protein, <i>MED27</i> codes for a component of the mediator complex	<i>RAPGEF1</i> could trigger a signalling cascade that is involved in apoptosis and cell transformation. <i>MED27</i> functions as a coactivator for RNA polymerase II-dependent genes.	Role of both genes is unknown. However, eosinophils from asthma patients show lower methylation and transcriptional signatures in whole blood indicate increased activation of the eosinophils, which could be an early marker of wheeze.	Childhood-onset asthma (4/5 years old and 8 years old)
cg19764973 (91)	Whole blood, purified eosinophils, respiratory epithelial cells	<i>STX3</i> , <i>MIRPL16</i>	Chr11q12	<i>STX3</i> codes for a transmembrane protein of the epithelial cells. <i>MIRPL16</i> codes for a subunit protein of the mitochondrial ribosome	<i>STX3</i> forms clusters on the epithelial membrane and maintains polarity across the membrane. <i>MIRPL16</i> contributes to protein synthesis within the mitochondrion	Role of both genes is unknown. However, eosinophils from asthma patients show lower methylation and transcriptional signatures in whole blood indicate increased activation of the eosinophils, which could be an early marker of wheeze.	Childhood-onset asthma (4/5 years old and 8 years old)
cg01445399 (91)	Whole blood, purified eosinophils, nasal respiratory epithelial cells	<i>LOC339524</i> (<i>LINC01140</i>)	Chr1p22	Long intergenic non-protein coding RNA	Unknown	Unknown. However, eosinophils from asthma patients show lower methylation and transcriptional signatures in whole blood indicate increased activation of the eosinophils, which could be an early marker of wheeze.	Childhood-onset asthma (4/5 years old and 8 years old)
cg13835688 (91)	Whole blood, purified eosinophils, nasal respiratory epithelial cells	<i>SLC25A25</i> , <i>PTGES2</i>	Chr9q34	<i>SLC25A25</i> codes for a calcium-binding carrier in the inner mitochondrial membrane. <i>PTGES2</i> codes for a membrane-associated prostaglandin E synthase.	<i>SLC25A25</i> probably mediates the transport of adenyl nucleotides. The precise function of <i>PTGES2</i> is unknown.	Unknown. However, eosinophils from asthma patients show lower methylation and transcriptional signatures in whole blood indicate increased activation of the eosinophils, which could be an early marker of wheeze.	Childhood-onset asthma (4/5 years old and 8 years old)

CpG-site	Cell type	Closest gene	Genomic location	Gene codes for	Function of the protein	Proposed disease mechanism in preschool wheeze	Associated phenotype
cg21486411 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>CLNS1A</i>	Chr 11q14	Protein in plasma membrane	CLNS1A functions in multiple regulatory pathways and functions as a chloride current regulator	Although lower DNA methylation is associated with increased childhood-onset asthma risk, the mechanism is unknown	Childhood-onset asthma
cg16792002 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>MAML2</i>	Chr 11q21	Protein within the mastermind-like family of proteins	MAML2 is involved in Notch signalling. The Notch pathway could increase airway inflammation in adult asthma(157)	Higher methylation of this site associates with lower expression of the gene and therefore could contribute to a protective risk against wheezing.	Childhood-onset asthma
cg13427149 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>GPATCH2</i>	Chr 1q41	Nuclear factor	Can enhance the ATPase activity	Although lower DNA methylation is associated with increased childhood-onset asthma risk, the mechanism is unknown	Childhood-onset asthma
cg17333211 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>SCOC</i>	Chr4q31	Short coiled-coil protein in the Golgi apparatus	Positive regulator of amino acid starvation-induced autophagy.	Although lower DNA methylation is associated with increased childhood-onset asthma risk, the mechanism is unknown	Childhood-onset asthma
cg13289553 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>SUB1</i>	Chr5p13	Protein located in the nucleus	Enables DNA binding activity and is involved in RNA polymerase II promoter clearance	Although lower DNA methylation is associated with increased childhood-onset asthma risk, the mechanism is unknown	Childhood-onset asthma
cg07156990 (93)	Blood, nasal respiratory epithelial cells, eosinophils	<i>WDR20</i>	Chr 14q32	Protein located in the nucleus and cytoplasm	Regulator of deubiquitinating complexes.	Higher methylation of this site associates with lower expression of the gene and therefore could contribute to a protective risk against wheezing.	Childhood-onset asthma

Abbreviations: *HK1*: Hexokinase 1; *CLMN*: Calmin; *RAPGEF7*: Rap guanine nucleotide exchange factor 1; *MED27*: Mediator Complex Subunit 27; *STX3*: Syntaxin 3; *MRPL16*: Mitochondrial ribosomal protein L16; *LINC01140*: Long Intergenic Non-Protein Coding RNA 1140; *SLC25A25*: Solute Carrier Family 25 Member 25; *PTGES2*: Prostaglandin E Synthase 2; *CLNS1A*: Chloride Nucleotide-Sensitive Channel 1A ; *MAML2*: Mastermind Like Transcriptional Coactivator 2; *GPATCH2*: G-Patch Domain Containing 2; *SCOC*: Short Coiled-Coil Protein; *WDR20*: WD Repeat Domain

Table 2.

Summary of Non-Invasive Biomarkers for Preschool Wheeze.

Biomarker	Key Points	Limitations
Allergen Sensitization	<ul style="list-style-type: none"> - Major risk factor for asthma development. - Present in ~40% of preschool wheezers. - Strong predictor of ICS response, especially with eosinophils > 300/μL. - Used in the PARK trial to select high-risk children. 	<ul style="list-style-type: none"> -Not all preschool wheezers are sensitized. -Early testing may not reflect future allergic status.
Blood Eosinophils	<ul style="list-style-type: none"> - Marker of airway inflammation. - Elevated in persistent wheezers; predicts asthma persistence. - Cutoffs (150–470 cells/μL) linked to exacerbation risk and hospitalization. 	<ul style="list-style-type: none"> -Cutoff values are not well standardized for preschool children.
FeNO	<ul style="list-style-type: none"> - Correlates with airway hyperresponsiveness and atopy. - High FeNO at age 4 linked to persistent wheeze and asthma diagnosis. - ERS recommends > 25 ppb in children > 5 years. - Utility in preschoolers is still under investigation. 	<ul style="list-style-type: none"> -Collection maneuver is hard for younger children to perform with accuracy. -Measurements are affected by viral infection and diet.
VOCs	<ul style="list-style-type: none"> - Reflects metabolic changes in breath. - Can distinguish asthma vs. transient wheeze with high accuracy. - Potential for early diagnosis and phenotype differentiation. 	<ul style="list-style-type: none"> -Collection maneuver is hard for younger children to perform with accuracy. -Measurements are affected by diet and environmental exposures.