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Childhood asthma- pathogenesis and phenotypes

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Key words

Asthma, children, pathogenesis, phenotypes, endotypes, inflammation, microbiome.

Take home message

Complex interactions between viral infections, atopy, the microbiome, preterm birth, infant weight gain, environmental exposures and genetic susceptibility influence the development of wheezing illness and asthma in children.

Abstract

In the pathogenesis of asthma in children there is a pivotal role for a type 2 inflammatory response to early life exposures or events. Interactions between infections, atopy, genetic susceptibility, and environmental exposures (such as farmyard environment, air pollution, tobacco smoke exposure) influence the development of wheezing illness and the risk for progression to asthma. The immune system, lung function and the microbiome in gut and airways develop in parallel and dysbiosis of the microbiome may be a critical factor in asthma development. Increased infant weight gain and preterm birth are other risk factors for development of asthma and reduced lung function.

The complex interplay between these factors explains the heterogeneity of asthma in children. Subgroups of patients can be identified as phenotypes based on clinical parameters, or endotypes, based on a specific pathophysiological mechanism. Paediatric asthma phenotypes and endotypes may ultimately help to improve diagnosis of asthma, prediction of asthma development and treatment of individual children, based on clinical, temporal, developmental or inflammatory characteristics. Unbiased, data-driven clustering, using a multidimensional or systems biology approach may be needed to better define phenotypes. The present knowledge on inflammatory phenotypes of childhood asthma has now been successfully applied in

the treatment with biologicals of children with severe therapy resistant asthma, and it is to be expected that more personalized treatment options may become available.

Introduction

Asthma is a multifactorial disease and although the underlying mechanisms are not fully understood, it has become clear that genetic vulnerability, atopy, respiratory infections, the lung and gut microbiome and environmental factors all play a role in asthma inception and pathogenesis. The complex interplay between all these factors explains the heterogeneity of asthma in children and supports a possible benefit of stratified precision medicine, in contrast to a 'one-size-fits-all' approach. Subgroups of patients can be identified based on clinical or biomarker criteria, often referred to as phenotypes. Clinical phenotypes may be helpful in diagnosis, prediction and treatment of children with wheezing illness or asthma. However, in real-life practice, there is a large heterogeneity and variability of phenotypic expression, meaning that phenotypes often overlap. The concept of endotypes has been introduced to understand how much each component of asthma pathophysiology contributes to the symptoms for each child in order to identify 'treatable traits' and enable individualised therapy. [1,2] Endotypic characterization has been proven particularly useful in the treatment of children with severe therapy resistant asthma. [3]

Recently, unbiased, data-driven clustering, using a multidimensional or systems biology approach, has been used to define phenotypes. This combines different data domains that include symptoms, psychosocial aspects, lung function, inflammatory parameters, exposures, metabolomics, epigenomics, transcriptomics, and fluctuation-based clustering. [4-9] By comparing several paediatric clustering studies and investigating phenotype stability during childhood some consistent patterns in phenotypes have emerged. [10] In this review we first aim to unravel the interplay between pathophysiology, lung and immune development and early life risk factors such as exposure to air pollution, viral infections, atopy, preterm birth, weight gain

and the microbiome in the development of asthma in children. In a further step, we aim to disentangle the complexity of paediatric asthma phenotyping with a focus on clinical relevance.

Early life risk factors in asthma pathogenesis

Childhood asthma is a complex disease with a multitude of host and environmental factors which contribute to the evolvement of the disease process. The relative contribution of each individual involved factors is typically small, however their interactions during lung functional growth and the development of the immune system may eventually lead to different airway disease phenotypes. Here we summarize risk factors and proposed mechanisms related to the early origins of asthma in childhood (Figure 1).

Microbes, allergen sensitisation and asthma susceptibility

Viral infections, in particular with human rhinovirus (HRV) and respiratory syncytial virus (RSV), and allergen sensitisation in early life are associated with asthma inception. [11,12] Thirty-two % of infants that had been hospitalized with severe RSV infection had developed allergen sensitization by 3 years of age, compared to only 9% of age and sex matched controls. [12] More severe illness induced by RSV (but also HRV) is associated with the development of asthma and increased risk of atopy and asthma continued to 18 years of age. [13] Interestingly, despite the link between viral infections and asthma inception, immunoprophylaxis with palivizumab against RSV did not reduce asthma diagnoses at age 6 years, although wheezing symptoms in preschool children were reduced. [14] There is also strong evidence that RSV-induced bronchiolitis can damage the airways to promote airway obstruction and

recurrent wheezing. [15] Still, the direct causal link between early RSV infection and allergen sensitisation, and which of these comes first in the context of asthma inception, remains uncertain. For HRV, data from a high risk birth cohort demonstrated that allergic sensitisation preceded HRV induced wheezing. [16] However, the direction of causation when considering an unselected population is unclear. Studies using murine models and in vitro studies in humans are being undertaken to understand a causal link. [17-20]

It is also apparent that genetic susceptibility is important in determining asthma outcomes following viral infection. Data from two independent, high-risk birth cohorts (at least one atopic parent) has shown that children with the highest risk for early onset asthma were those who had HRV-related wheezing illnesses in early life and were carriers of an at-risk allele in the ORMDL3 gene locus. [21] Important insights on the critical role of gene-environment interactions in determining outcome can be derived from farmyard studies. Children with at-risk alleles in the ORMDL3 gene locus had strong protection against asthma if raised on animal farms. [22] The biological modifying mechanisms associated with the 17q21 locus are still incompletely understood. Interestingly, in addition to being raised on a farm, they modify the effect of a series of risk factors associated with recurrent airway disease such as susceptibility to viral infections, breastfeeding or tobacco smoke exposure. [22] These modifications are also likely to involve several genes in the region, including ORMDL3, GSDMB, and several cell types, including immune and airway epithelial cells. This suggests that, in the absence of high levels of microbial exposure from the farmyard environment in early life, ORMDL3 enhances the clinical expression of wheezing associated with viral infection. In contrast, ORMDL3 risk alleles seem to provide protection from wheezing in the presence of favourable microbial exposures.

Undoubtedly, for both HRV and RSV, interactions between viral infection, genetic susceptibility, and environmental exposures influence the development of wheezing illness and the risk for progression to asthma.

Airway and gut microbiome and asthma onset

The last decade many studies have shown that the airway and gut microbiome may be important in asthma development and that dysbiosis of gut and lung microbiome and delayed maturation of immune responses and airway microbial communities are related to the early development of asthma. [23-26] Asthma and preschool wheezing have been associated with a microbiota characterized by lower diversity and an abundance of Moraxella, and reversely, increased bacterial diversity has been positively associated with protection. [24] [27]

The healthy lung microbiome is characterized by bacteria belonging to the phyla Bacteroidetes, Actinobacteria, and Firmicutes. [28] Exogenous factors can affect the natural lung microbiota composition positively (farming environment) or negatively (allergens, air pollutants). The importance of environmental exposures within a critical window in early life, during which immune maturation occurs and the microbiome develops, is becoming increasingly apparent. In mice, the development of airway eosinophilia and hyperresponsiveness after house dust mite (HDM) exposure at a young age appeared to be dependent on the airway microbiome. [29] Additionnally, in pups exposed to HDM, inhaled Acinetobacter iwoffii (a bacteria found in the protective farmyard environment) resulted in protection from airway eosinophilia and hyperresponsiveness.[30] The development and maturation of the respiratory microbiome in early life depends on exposures in the first few hours including delivery mode, and the environment in the following 4 to 5 months. [31-33]

The composition of indoor dust and bacterial exposure at 2 months of age have been associated with asthma development by 10 years. [34] In dust samples from living rooms of infants at 2 months of age, bacterial richness was inversely associated with asthma. Lactococcus genus was a risk factor for asthma, while the abundance of 12 bacterial genera, predominantly from the Actinomyces species were associated with lower asthma risk. [34] The pattern of microbes that were associated with protection from asthma are similar to those found in the protective farmyard environment. [35] Farm studies have linked both the environmental and the host microbiome to asthma. [24,27] Except for bacterial diversity, also low fungal diversity in house dust mite was associated with development of asthma and increased exposure to dust yeast in early-life may be protective against asthma and allergy. [36,37]

Dysbiosis of the gut microbiota results from several external influences including smoke exposure, antibiotics, or diet, and this has been associated with altered systemic and local immune responses, including inflammatory changes in the lung. [28,38] The exact mechanisms of communication in this gut-lung axis are not clear but are partially mediated by bacterial metabolites, exerting immune responses in remote parts of the body such as the lungs. [39] The relation between diversity in the gut microbiome and asthma development has been shown in several studies. Also, exposure to antibiotics in early life is associated with an increased risk of asthma development [40] and this effect might be explained by an effect of antibiotics on the gut microbiome.

The use of nonspecific immunomodulators derived from bacterial respiratory tract pathogens is gaining increasing attention. [41] In a retrospective study in children with recurrent respiratory tract infections oral OM-85, which consists of alkaline lysis of 21

bacterial strains of respiratory tract pathogens, resulted in a significantly lower frequency of respiratory tract infections, wheezing episodes and intake of antibiotics.[42] A randomised controlled trial of sublingual MV130 (a mixture of 6 inactivated bacteria) has shown reduction in preschool wheeze attacks compared to placebo. [43]

We thus have increasing evidence that the development and maturation of the lung and gut microbiome may affect the risk of asthma development. Whether early manipulation of the microbiome can reduce the risk of asthma remains to be shown.

Early life nutrition and asthma onset

Several studies suggest that the onset of asthma may be influenced by early life nutrition including breast feeding. Systematic reviews have shown a consistent protective effect of ever being breast fed on later wheezing and asthma, which seemed to wane off in adolescents. . [44-47] Most studies suggest that longer duration of breastfeeding lowers the risk of asthma compared to a shorter duration. [44-47]

How breastfeeding exactly modifies the risk of asthma development is not fully clear but effects on developing immune system, microbial colonization and the integrity of the gut mucosal barrier may all play a role. [48]

There is a lack of evidence for partially or fully hydrolized milk formula in preventing asthma, even in high-risk infants. [49]

Vitamin D has been implicated as a protective factor for viral immunity in the lower airways, and may have favourable effects in asthma. Data on vitamin D status and vitamin D supplementation of the mother during pregnancy on asthma risk in the offspring are conflicting, with a majority of systematic reviews and meta-analyses suggesting that supplementation of vitamin D during pregnancy reduces the risk of

wheezing and/or asthma. [50-57] One meta-analysis showed a U-shaped association between dose of vitamin D supplementation during pregnancy and risk of wheezing/ asthma, suggesting that both low and high dosages of vitamin D may have negative effects.[58] The variable results of vitamin D supplementation may be explained by recent evidence that maternal 17q21 genotype has an important influence on the protective effects of prenatal vitamin D supplementation against offspring asthma/recurrent wheeze. [59] Infant supplementation with vitamin D did not affect the risk of asthma at school age. [60] Omega-3 fatty acids have been assigned anti-inflammatory properties and supplementation of omega-3 fatty acids during pregnancy has shown a positive trend in reducing wheezing during the first years of life, but not asthma at schoolage. [52,61,62] Similarly, supplementation of omega-3 fatty acids during childhood did not reduce the risk of asthma. [62]

Early life exposure to pollutants and asthma onset

Epidemiological data support the link between even low-level exposure to air pollution during gestation and early life, and asthma development and impaired lung function growth.[63-66] The effects of weekly average exposure to particulate matter with an aerodynamic diameter less than 2.5 μm ($PM_{2.5}$) during pregnancy and infancy on asthma development were investigated in a birth cohort of 184,604 children in Taiwan. [67] Increased exposure to $PM_{2.5}$ during gestational weeks 6 to 22 and between 9 to 46 weeks after birth were significantly associated with an increased incidence of childhood asthma. The effect of air pollution on lung function is dose dependent and is more pronounced when children are exposed during early lung development. [64] Data from 3 separate cohorts in the US showed that declining levels of nitrogen dioxide (NO_2), $PM_{2.5}$ and PM_{10} were associated with improved lung

function growth in children with and without asthma. [68] In central London, UK, a sequential annual cross-sectional study of 2164 children aged 8-9 years between 2009-10 and 2013-14 was undertaken following the introduction of London's Low Emission Zone in 2008. [69] The percentage of children living in areas exceeding the EU limit value for annual NO_2 exposure fell from 99% in 2009 to 34% in 2013. FVC was inversely correlated with annual NO_2 and PM_{10} exposure, but there was no association between postbronchodilator FEV_1 and annual residential pollutant attributions. Although pollution exposure fell dramatically, there was no significant impact on FVC over the 5 years study, suggesting the reduced lung volumes for age were a reflection of much longer term exposure and would fit with data from mechanistic models which highlight particular periods of vulnerability to even low-level exposures during pregnancy and immediately postnatally. [64,65,67]

Observations in a mouse model suggested that postnatal concurrent exposure to both inhaled HDM and diesel exhaust particles (DEPs) result in more severe and less corticosteroid sensitive airway disease. [70] Of note, murine models of maternal DEP exposure before and/or during pregnancy, with subsequent allergen and DEP exposure in early neonatal life, showed development of more severe allergic airway hyperresponsiveness in offspring compared to allergen exposure alone. [71-73]

Obesity, early life weight gain and asthma development

The relationship between obesity and asthma is a complex one, and bidirectional since asthma increases the relative risk of obesity by 1.5–1.7 fold, but obesity is also an independent risk factor for asthma. [74-76] A meta-analysis which included 18 prospective studies, calculated a 20% risk increment for asthma in overweight children and a 40% risk increment in obese children. [77]

Longitudinal assessments of birth weight and BMI to the age of 17 were used to investigate associations of adiposity with asthma in >6,000 children in the Taiwan Children Health Study. [78] Mendelian randomisation analysis revealed that the critical period during which adiposity predicted childhood asthma outcomes was before the age of 6 years. This suggests that the preschool years are a critical window to focus on healthy growth to influence later asthma. A similar analysis in the Avon Longitudinal Study of Parents and Children (ALSPAC) also showed that higher BMI in early life increased the risk of asthma by age 7 years. [79] The rate of weight gain in the first few years of life may therefore be an important factor for asthma development. [80] In a meta-analysis of almost 25.000 children infant weight gain was associated with an increased risk of asthma and lower FEV_1/FVC ratio and forced expiratory volume after exhaling 75% of vital capacity (FEF_{75}). Data from the Boston birth cohort has shown extremely rapid weight gain during the first 4 and 24 months of life were each associated with increased risks of asthma, even after adjusting for birth weight and preterm birth. [81] Although epidemiological data are increasingly pointing to the preschool years as the susceptible period in determining long term asthma outcomes, the mechanisms underlying the effect of weight gain on asthma susceptibility are unknown. Of interest, a post-hoc analysis of 3 randomised trials of ICS therapy in children aged 2-5 years with preschool wheezing has shown that overweight or obese children in the placebo arms had more exacerbations and symptoms compared to normal weight children. [82] Overweight-obese children that received ICS were as responsive to therapy as normal weight children, suggesting the mechanisms of “steroid resistance” in obese children with asthma later in childhood may not apply during the preschool years.

Prematurity and asthma development

It is well known that preterm birth and low birth weight are important early life risk factors for lower lung function later in life, and for the development of asthma.[80] In a meta-analysis of 20 studies, children born preterm had a mean 7.2% lower FEV1 predicted compared to term born controls, and in children diagnosed with bronchopulmonary dysplasia the mean FEV1 was even 16.2% lower. [83] Associations between lung function and gestational age (GA) are present across the full range of GA. [80] Even late preterms (born after 34-36 weeks GA) had a significant decreased FEV1 compared to term born controls. Recent work showed that air pollution exposure during pregnancy had a more pronounced deteriorating effect on postnatal lung function in infants born prematurely, indicating that certain premorbid susceptible populations may react differently to environmental stimuli in early life. [84] The negative impact on lung function has also been shown for children born small for GA, although the effect is slightly different: children born with a younger GA had lower FEV1 and FEV1/forced vital capacity (FVC) ratio, while children born small for GA had lower FEV1 but higher FEV1/FVC ratio. [80] This suggests smaller lungs with less obstruction in children born small for GA. After adjusting for lung function, preterm birth and low birth weight are also predictors of later asthma. [80,85] Again, this effect has been shown for the full range of GA and even early-term born children (37.0 to 38.6 weeks GA) had an increased risk of asthma with an adjusted odds ratio of 1.20 (95%CI 1.17-1.23) compared to full term born children. [85] This increased risk of asthma after preterm birth continues into middle-age. [86] Additionally, preterm born children are at increased risk of severe bronchiolitis with RSV or rhinovirus, infections that may further increase the risk for subsequent asthma. [87,88] The mechanisms underlying the increased risk on lower lung function and asthma

development in premature born children are not fully understood. Disrupted development of airways, together with adverse exposures such as mechanical ventilation, oxidative stress and inflammation have been suggested to cause structural damage and a decrease in airway calibre, fewer and simplified alveoli and increased lung fibrosis. [89,90] In addition, adverse events in early life such as exposure to air pollution or tobacco smoke, and viral infections and preschool wheeze may lead to ongoing disease and reduced lung function growth. [89] Also, microbial dysbiosis in the gut microbiome, with a relative abundance of Proteobacteria and Firmicutes, and decreased Lactobacilli were reported with bronchopulmonary dysplasia (BPD) progression. [91] Delivery by caesarean section and postnatal antibiotic use influence this dysbiosis.

Data from Australia showed that the deficits in lung function in children with BPD increased between 4 and 12 years of age and that children with signs of inflammation on chest CT scan, such as bronchial wall thickening, had the worst lung function trajectories. [92] This suggests that there is an ongoing, inflammatory process in the lungs and airways of children with BPD. This ongoing disease is different from the typical eosinophilic inflammation as seen in asthmatic children as the fraction of exhaled nitric oxide (FeNO), as a surrogate for eosinophilic inflammation, in children born preterm with or without BPD were similar to controls. [93,94] Indeed, two studies showed evidence of neutrophilic inflammation and oxidative stress in the airways of preterm born adolecents. [95,96] Therefore, preterm born children with or without BPD may display a separate ‘asthma’ phenotype with various degrees of, partly reversible, obstructive and restrictive lung function abnormalities and signs of neutrophilic inflammation, which may not respond well to ICS.

In summary, immune responses in early life to viruses, allergens and/or pollution have a tendency towards exaggerated type 2 immunity, especially in susceptible children (Figure 1). However, it remains to be seen if this skewing can be “rescued” if the child is placed in a protective environment which favours a more diverse and mature airway and/or gut microbiome. The maturing immune system and airway microbiome appear to progress in parallel as there is a tight link between environmental exposures and microbial interactions and resulting immune responses. Obesity and rapid weight gain in the first few years of life may be other important factors that determine asthma outcomes. Last, preterm birth is a risk factor for later asthma and for reduced lung function.

Phenotypes of childhood asthma

The complex interplay between genetic background and early life risk factors in asthma development leads to heterogeneity of asthma phenotypes in children. Therefore, the second aim of this review is to disentangle the complexity of paediatric asthma phenotypes, defined as one-or-more observable or measurable clinical properties, [97] and ‘endotypes’ defined by a specific mechanism. Clinical phenotypes are related to inflammatory endotypes (e.g. childhood onset allergic asthma and T2 high inflammation), but there may be more endotypes within a phenotype. [2,98,99] Specific to paediatric wheezing disorders are the age-dependent and interacting relationships between clinical phenotypes (disease severity, temporal symptom pattern), type of inflammation, environmental triggers (e.g., viral infections, allergens, pollutants), response to treatment and comorbidities. In daily clinical

practice phenotypes are most useful in four domains: diagnosis, prediction, therapy and prevention and the stability of disease and phenotype (Figure 2).

Diagnosis

The most obvious phenotyping has been based on symptoms, where symptom severity (mild to moderate versus severe), the presence or absence of atopy, exacerbation rate, symptom pattern (episodic, intermittent, recurrent/chronic) and triggers (infections, allergens, exercise) are distinct features. [100-104] Two archetypical groups have thus been identified: children with transient, often episodic symptoms and those in whom recurrent wheezing persists beyond the first 6 years of life. Transient wheezing disorders are frequently observed in the first years of life, typically following viral infections and characterized by an episodic symptom pattern and symptom-free intercurrent intervals. This group often does not receive an asthma diagnosis. Children with typically recurrent or chronic wheeze have symptoms often triggered by multiple environmental factors (viruses, allergens, pollutants) in the first years of life, but tend to persist into later childhood and remain stable after the age of six. [105]

Prediction

For the purpose of prediction, research has focused on the prospective identification of temporal phenotypes (transient, remittent, persistent wheezing or asthma) (Figure 2). Such temporal phenotypes have been identified using cohort studies but unfortunately, temporal phenotypes can only be reliably assigned in retrospect, and this limits their clinical application. [106-108] In order to find ways to prevent the development of persistent asthma it is important to examine the development of

pathophysiological endotypes. Although the mechanisms of transition into adulthood are still a matter of research, some underlying pathophysiological mechanisms such as traits associated with tracking of poor lung function after impaired lung development, or with the development of allergy, traits exhibiting features of metabolic syndrome with low-grade inflammation and oxidative stress (obesity, pollutants) or traits associated with microbial interactions (microbiome, viral infections) have been identified. Although approximately 30–50% of these recurrent asthma forms persist into adulthood, a significant proportion of children experience intermittent symptom patterns or symptom remission during adolescence, in particular male patients and children with milder symptoms and a low degree of atopy. [109] Nevertheless, some evidence suggests that despite clinical symptom remission, these patients may have persisting lung function impairment, bronchial hyper-responsiveness, and airway inflammation and remodelling as an expression of a persistent underlying subclinical disease process in adolescence. [110-112] In order to reliably identify such trajectories, research in early endotype-specific risk factors and biomarkers is needed. Such traits and associated risk factors are increasingly better described. For example, environmental air pollution, tobacco smoke exposure, premature birth, nutritional effects and chronic airway inflammation have been shown to be related to impaired lung function growth and recurrent asthma symptoms. [113] Another such trait may be related to allergic pathways, whereby studies have shown trajectories with different types of sensitisation. [114,115] However, allergic sensitisation and its relation to asthma progression is heterogeneous. [115-118] Some features have been shown to be clearly associated with asthma trajectories—these are multiple, high or early sensitisations and also specific sensitisation to dog, cat or horse allergens, which are associated with later onset of asthma. [119] While such

trajectories offer personalised options for prevention or treatment, early endotype-specific biomarkers or multi-omic profiles are needed. Ideally, these should be sensitive to the onset of asthma either early on or prior to the disease process.

Therapy and prevention

Asthma phenotyping has the potential to improve and personalize asthma management, either by better targeting of current treatments, or by new treatment approaches. [99,120] An important consideration here is that current paediatric asthma treatment largely relies on a simple regimen of first-line asthma drugs that have proven to be effective, safe and cheap. [121] Although these drugs do improve symptoms and lung function, ICS do not seem to influence lung function trajectories in children with asthma. [122]

Personalized asthma treatment has been successfully applied to children with severe therapy resistant asthma (STRA), a phenotype which does not respond to the standard high doses of ICS (>800 µg budesonide or equivalent). [123] STRA needs to be separated from 'difficult to treat asthma', where poor asthma control is mainly a result of poor adherence, poor control of comorbidities (e.g., obesity, allergic rhinitis, psychosocial distress) or contextual circumstances (e.g., familiar, educational, societal). Cohort studies have identified four sub-phenotypes in STRA: late-onset asthma with normal lung function, and 3 sub-phenotypes of early-onset atopic asthma with normal lung function, mild airflow limitation and, finally, severe airflow limitation. [124] If patients remain stable within such clusters over time, such pathological phenotypes may help guide add-on therapy. In STRA, a good example of endotype-specific treatment is the introduction of biologicals for the treatment of atopic asthma. At present, omalizumab (anti-IgE), mepolizumab (anti-IL5) and

dupilumab (anti-IL4 and anti-IL13) are approved for use in children from the age of 6 years (omalizumab and mepolizumab) and 12 years (dupilumab), with omalizumab having the longest track record. [3,125] As biologicals interfere with a specific component of the inflammatory cascade, patients are selected on the basis of their inflammatory phenotype, with quantitative cut-offs. Only children with the allergic, eosinophilic phenotype are eligible for such treatment, with prescription algorithms depending on phenotype. Evidence for clinical effectiveness of biologicals in children older than 12 years of age has recently been summarised. [123]

Disease phenotype and stability

The terms ‘controlled’, ‘partly controlled’, ‘uncontrolled’ or ‘brittle’ asthma describe clinical symptom patterns, asthma control and exacerbation risks. In clinical practice such terminology is largely used in a qualitative or semiquantitative manner and often does not support precise treatment decisions. Efforts to quantify asthma control in an observer-independent manner (e.g. by using asthma control tests or questionnaires) have now become established tools for guiding asthma treatment. However, quantifying asthma exacerbation risk or risks for phenotype stability and persistence of asthma remains a challenge in childhood asthma. Recent studies have used observer-independent clustering methods to characterise symptom patterns associated with asthma, with a potential use in telemonitoring screening tests. [126] It has been hypothesised that the dynamics of weekly fluctuations of symptoms (i.e. how fast the lung recovers from viral infection) in the first year of life are related to an asthma phenotype. The dynamics of symptom fluctuations—and not the number of symptomatic weeks—could identify a cluster of infants with a higher risk for subsequent persistent wheeze in preschool age. [127]

Others have used biomarkers such as regular measurements of lung function or FeNO to identify clusters of children with asthma in an observer-independent manner (dynamic phenotypes, fluctuation phenotypes). [9,128] Of particular interest are novel approaches using clustering methods that consider a time-series of fluctuations, e.g. in daily adherence with inhaler treatment. [129] Although the techniques are all in their infancy, data from adult asthmatics show that such fluctuation-based methods of telemonitoring using novel machine-learning methods have the potential to characterise asthma stability and exacerbation risks in an observer-independent manner. [130-133] Assessment of daily fluctuations of airway inflammation have become feasible with FeNO; FeNO shows a high degree of day-to-day fluctuation. [134] While single values will not reflect the entire inflammatory disease process, recent evidence has shown that a series of daily FeNO values is long-range correlated and related to exacerbation risks in children. [128,135]

Inflammatory phenotypes

Chronic inflammation of the airways plays a central role in the pathogenesis of asthma and anti-inflammatory treatment with inhaled corticosteroids (ICS) is the treatment of choice. The pattern of inflammation is determined by several factors including age, aeroallergen sensitisation, airway infection (viral or bacterial) and disease severity.

In preschool children (< 5 years) with recurrent wheezing three recent unbiased analyses have shown distinct clusters of inflammation in the lower airways with predominant eosinophila associated with aeroallergen sensitisation, or neutrophilia associated with bacterial and/or viral infection. [136] The findings were independent of clinical phenotypes and symptom pattern. [137] A significant proportion of children in

this age group therefore does not have lower airway eosinophilia, and the inflammatory heterogeneity helps to explain why many younger children with recurrent wheezing do not respond to ICS. [136] We now have evidence to support the need for more objective inflammatory phenotyping in this age group prior to making management decisions. In preschool children blood eosinophils correlate with airway eosinophils, and may be helpful to guide ICS treatment. The INFANT trial has supported this by showing that preschool children with recurrent wheezing and aeroallergen sensitisation with blood eosinophils > 300 cells/ µl were differential responders to ICS. [138] However, what is still lacking are non-invasive biomarkers that will identify the neutrophilic/ infection phenotype. There is some observational data to support targeted antibiotic therapy, including a recent study showing that low levels of TNF-α and IL-10 and high levels of CCL22 in nasal lining fluid of children with preschool wheeze predicted favorable treatment response to azithromycin during acute epsiodes of asthma-like symptoms. [139] [140-143] However, definitive interventional trials are lacking.

The majority of school-age children with mild-moderate asthma have allergic, eosinophilic airways disease associated with type 2 immune responses, including elevated interleukin IL-5 and IL-13.[144] This inflammatory phenotype is supported by indirect measures of inflammation, including exhaled nitric oxide, a T2 biomarker, which is elevated in these patients. [145] Therefore, the majority of school-age asthma is responsive to ICS and can be controlled. If there is persistent poor control with continuing escalations in treatment, the most common explanation is failure to address the basics of management, including adherence to ICS therapy, and appropriate inhaler device or technique. Children with poor control because of unresolved modifiable factors have ‘difficult-to-treat asthma’. [3] They commonly have

a steroid sensitive airway eosinophilia which improves when the basics are addressed, together with evidence of improved lung function, reduction in ICS dose and fewer exacerbations up to 5 years later. [146,147]

Most evidence relating to airway inflammation in childhood asthma is biased towards severe disease, because invasive diagnostic techniques are often impossible in children with milder disease. Severe asthma in children is predominantly associated with persistent eosinophilic airway inflammation which may be relatively resistant to treatment with ICS. [3] [146] Interestingly, airway eosinophilia persisted despite reduced levels of interleukin IL-5 and IL-13, that are supposed to drive allergic asthma. The absence of these cytokines, together with persistence of eosinophilia in STRA has led to the hypothesis that innate mediators such as IL-33, which appear relatively steroid resistant, may dominate the immune response in STRA. [148,149] Some studies have identified a 'type-2-low' asthma endotype, associated with airway neutrophilia. [150-154] However, IL-4, 5 and 13, the signature type 2 cytokines, are very steroid sensitive, and thus steroid-treated patients are unlikely to have detectable levels. Hence, type-2-low asthma could be explained by suppression of type-2 cytokines by corticosteroids. [155] Although a neutrophilic phenotype seems uncommon in school-age asthma, analysis of cytokines in broncho-alveolar lavage fluid (BALF) from children with severe asthma has shown a mixed picture of type 2, type 1 (characterized by IL-12, tumor necrosis factor-α and interferon-γ) and T-helper17 (characterized by IL-17 and granulocyte-colony stimulating factor) which are associated with BALF neutrophilia. [154] Children with neutrophilic inflammation were younger compared to those with eosinophilic or non-neutrophilic inflammation (median age 6, 11 and 10 years, respectively) suggesting neutrophilic disease is more common in preschool asthma. [137,154]

It is therefore unclear, especially in the context of STRA, whether or not type-2-low asthma is a distinct phenotype or merely represents concomitant bacterial infection or suppression of type 2 inflammation by corticosteroids. [156] However some asthmatic children are non-atopic, and more mechanistic studies (characterisation of endotypes) are needed in this subgroup of children where therapeutic options are currently limited.

Little is known about the exact role of innate lymphoid cells type 2 (ILC2) in children with asthma. ILC2s in the airways are activated by cytokines derived form the epithelium, thymic stromal lymphopoietin, IL-25 and IL-33, and may recruit and activate eosinophils by IL-4, IL-5 and IL-13. Children with STRA had higher ILC2s compared to children with difficult to treat asthma, and it has been suggested that IL-33 contributes to the activation of ILC2 in STRA ; ILC-2s decreased after treatment with systemic corticosteroids. [146,149,157] There are no data on the role of ILC2s in children with mild-moderate asthma.

Current limitations in the clinical application of phenotypes and endotypes as a basis for treatment

Information on the stability of a phenotype is crucial before it can be considered as a guide to the management of asthma. Phenotypes should therefore be studied for reproducibility, feasibility and temporal stability. Such validation is often lacking, especially in children. With respect to viral wheeze (episodic, transient phenotype) versus multiple-trigger wheeze (recurrent, persistent phenotype), some years ago an ERS taskforce concluded that, in the absence of evidence in favour of specific treatments, and the variability in time within subjects, the two phenotypes should be treated similarly, based on the severity and frequency of symptoms. [158] [10,159,160].

Treatment guided by eosinophils in sputum has been shown to be beneficial in adults with asthma, but a single study in children with severe asthma showed no clear benefit. [161] [162] A study on sputum cytology in children with mild to severe asthma showed that 63% changed their inflammatory phenotype at least once a year, irrespective of asthma severity or treatment changes, which also limits the use of inflammatory phenotypes to guide asthma treatment. [163] Invasive diagnostic methods cannot be applied to most children with asthma, and there has been a keen interest in noninvasive biomarkers of specific biological processes of which FeNO as a marker of eosinophilic inflammation is a prominent example. [134] The introduction of FeNO in clinical practice has been preceded by decades of research including basic and methodological studies and many clinical trials of FeNO-guided treatment and other applications. Finally, meta-analysis of FeNO-guided treatment in children resulted in an average significant reduction of 37% in exacerbation risk, without effects on other aspects of asthma control. [164-166] Presently, FeNO monitoring is not recommended for routine use in clinical asthma care, but considered useful in selected patients. [167] Many more biomarkers in exhaled air, breath condensate, induced sputum, blood and urine that might be used to define the inflammatory phenotype in children have been studied in the past decades, often employing extremely sensitive assessments of substances of potential interest. Unfortunately, to date, none of these have proven feasible, and there is no evidence of benefit to clinical decision making. To better define phenotypes an unbiased, data-driven clustering, using a multidimensional or systems biology approach may be needed. For example, in a cohort of 300 children within the Severe Asthma Research Program unsupervised clustering identified 4 clusters of children with severe asthma with differences in clinical and pathophysiological characteristics. [168] Latent class

analysis in a population-based cohort revealed 4 asthma trajectories, that may imply different treatment approaches. [169] Other groups used multi-level learning approaches for predicting asthma phenotypes using high-dimensional biomarkers signatures including questionnaires, genotype, microarrays, Real Time-qPCR, flow cytometry, and cytokine data. [170] Similar approaches may be used to predict asthma development in young children, better define asthma phenotypes and pave the way for personalized treatments. At present such multi-dimensional strategies are hardly explored yet.

Conclusions

In the development of asthma, genes and gene-environment interactions play an important role and protective environments such as farmyards may offer clues to prevent asthma development in susceptible children. In this respect, the airway microbiome and the lung-gut microbiome axis offer interesting insights, as dysbiosis of gut and lung microbiome seems involved in the development of later asthma. Possible preventive measures also include prevention of preterm birth (e.g. by smoking bans), reduction of adverse exposures such as air pollution and focus on healthy lifestyle and prevention of excessive weight gain in the first 2 years. [171] Paediatric asthma phenotypes may help to answer questions on the optimal treatment of individual children and may be based on clinical, temporal, developmental or inflammatory characteristics. Phenotypes may be helpful in prediction of persistent asthma, identifying personalised options for prevention and to personalize treatment or disease modification of asthma. At present, already, inflammatory phenotypes guide the use of biologicals. Multidimensional approaches

integrating different high-dimensional biomarkers are hardly explored yet, but show promise for asthma prediction.

During recent years much progress has been made in unravelling the many factors involved in asthma development and pathogenesis. The next challenge is to translate this knowledge in preventive and therapeutic strategies to reduce the burden and improve the prognosis of asthma in children.

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Legends to figures

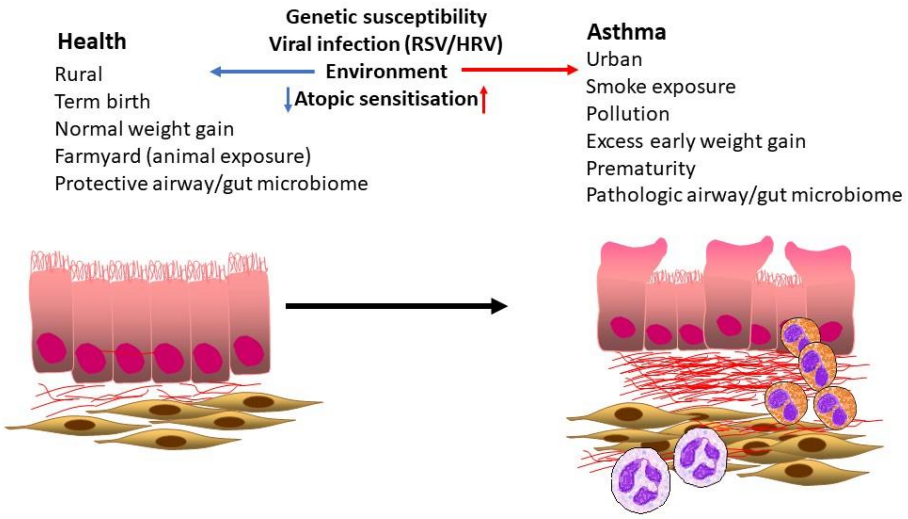

Figure 1

Overview of early life factors that increase or decrease the risk of asthma development.Genetic susceptibility, viral infections and atopic sensitization interact with environmental factors which may increase (living in an urban environment, exposure to cigarette smoke, air pollution, excess infant weight gain, preterm birth and disturbed airway/ gut microbiome) or decrease (living in a rural environment, term birth, normal infant weight gain, protective airway/ gut microbiome) the risk of development of wheezing and asthma.

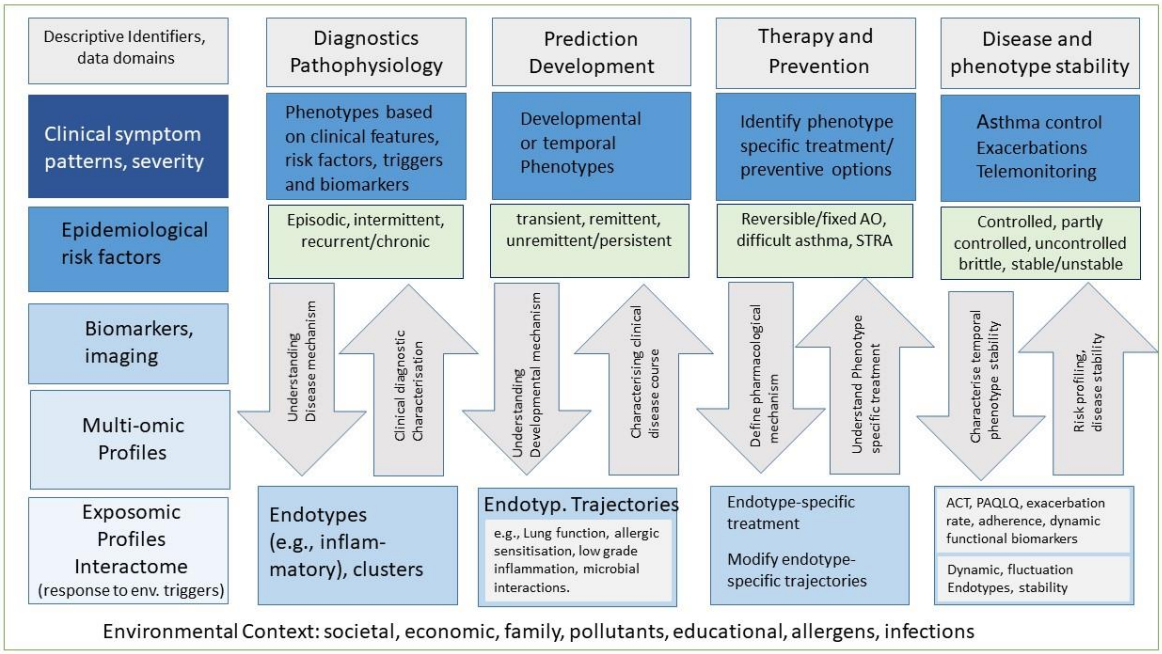

Figure 2

Overview on phenotypes and endotypes in childhood asthma. In daily clinical practice phenotypes are most useful in four domains: diagnosis, prediction, therapy and prevention and disease and phenotype stability.