Review

Bronchial hyper-responsiveness after preterm birth

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Educational aims

The reader will be able to

- Appreciate that bronchial hyper-responsiveness is a characteristic observed in a variety of lung diseases.
- Understand that bronchial hyper-responsiveness is likely to involve different causal mechanism in different lung diseases.
- Distinguish between different techniques used to test bronchial hyper-responsiveness and how preterm born survivors may respond to those tests.

ARTICLE INFO

ABSTRACT

Being born preterm often adversely affects later lung function. Airway obstruction and bronchial hyper-responsiveness (BHR) are common findings. Respiratory symptoms in asthma and in lung disease after preterm birth might appear similar, but clinical experience and studies indicate that symptoms secondary to preterm birth reflect a separate disease entity.

BHR is a defining feature of asthma, but can also be found in other lung disorders and in subjects without respiratory symptoms. We review different methods to assess BHR, and findings reported from studies that have investigated BHR after preterm birth. The area appeared understudied with relatively few and heterogeneous articles identified, and lack of a pervasive understanding. BHR seemed related to low gestational age at delivery and a neonatal history of bronchopulmonary dysplasia. No studies reported associations between BHR after preterm birth and the markers of eosinophilic inflammatory airway responses typically found in asthma. This should be borne in mind when treating preterm born individuals with BHR and airway symptoms.

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Introduction

Preterm birth is a poorly understood, relatively common and potentially serious early life event that can affect the entire life course of an individual. Respiratory complications are important, as early birth implies that gas exchange must take place in foetal and thus developmentally immature lungs. Symptoms often begin shortly after birth and manifest as grunting, tachypnea, chest wall retractions and increasing respiratory difficulty that can develop into respiratory failure. Unless appropriately treated, infant respiratory distress syndrome (IRDS) carries a high risk of death. Despite vast medical and technological advances, IRDS remains challenging also in contemporary neonatal intensive care units (NICUs). Prolonged periods of oxygen supplementation, ventilatory

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support and a number of intensive care measures are often required. Although necessary to preserve life, these interventions can paradoxically lead to life-long pulmonary injuries. Thus, in a large proportion of survivors, extrauterine development of immature foetal lungs combined with injuries from life-saving neonatal interventions can lead to the development of chronic lung disease of prematurity (CLD), particularly in infants born 'extremely preterm' (EP); i.e. before 28 weeks' gestational age (GA) [1]. CLD, often also called bronchopulmonary dysplasia (BPD), is commonly defined by requirement for supplemental oxygen treatment for more than 28 days of age, and further classified at 36 weeks GA into mild, moderate or severe BPD [2].

Most long-term follow-up studies after EP-birth and BPD report abnormal lung function, with variable degrees of airway obstruction, bronchial hyperresponsiveness (BHR), pulmonary hyperinflation and impaired gas diffusing capacity. Abnormalities tend to increase with increasing immaturity and increasing severity of BPD [1], and in recent studies, also with intrauterine growth restriction [3,4], underlining potential influences of antenatal factors [5]. Autopsy studies of infants dying from BPD reveal increased airway wall dimensions with smooth muscle hypertrophy and abnormal elastin and collagen distribution, compatible with the functional findings from follow-up studies [6,7]. Access to autopsy studies and pathological specimens after infancy is scarce, and the links between lung function, pulmonary structure and pathophysiology are unclear. For example, we do not know if lung disease after preterm birth represents a consequence of previous structural injuries, or a current active (inflammatory) airway disorder with bronchospasm playing an important role. This lack of understanding of the underlying mechanisms prevents rational and effective therapy. Fundamental in this context, is a better understanding of the phenomenon of bronchial hyper-responsiveness (BHR), reported from several follow-up studies of preterm born individuals. The purpose of this review is to give an overview of current knowledge pertaining to BHR in this vulnerable group of individuals (see Fig. 1).
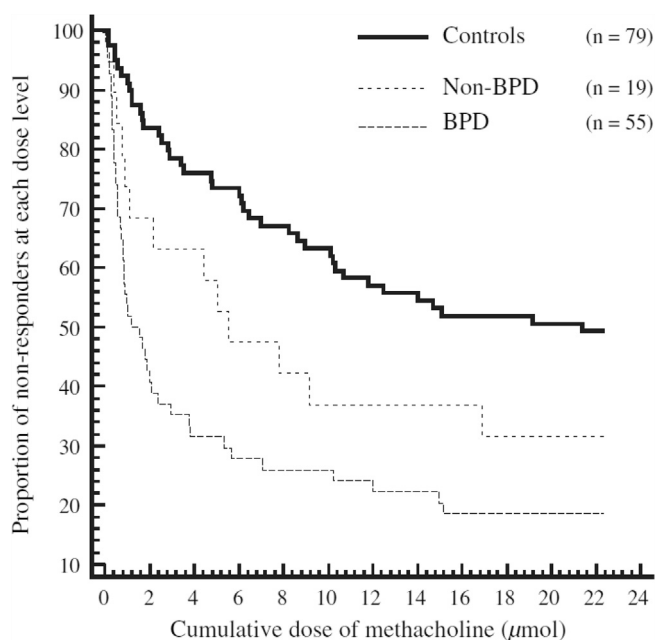

Fig. 1. Illustrating methacholine response in extremely preterm born subjects with and without BPD. Previously published: Halvorsen, T., et al. (2005). Characteristics of asthma and airway hyper-responsiveness after premature birth. <u>Pediatr Allergy Immunol</u> **16**(6): 487–494.

Bronchial hyper-responsiveness

Overview

BHR refers to excessive airway narrowing in response to a variety of inhaled stimuli. BHR is a common feature of asthma; however, with significant overlap with the responsiveness that can be found in non-asthmatic subjects. Thus, BHR is observed in almost all patients with asthma, but also in a significant number of individuals without respiratory complaints. It is customary to divide the stimuli applied to assess BHR into two categories: direct and indirect. Direct stimuli are pharmacological agents that act directly on specific receptors on the bronchial smooth muscle, with histamine and methacholine most commonly used. Indirect stimuli act via mediator release from inflammatory cells in the airway mucosa, and include exercise and eucapnic hyperventilation, hyperosmolar aerosols such as saline or mannitol, and the pharmacologic agent adenosine monophosphate. Data indicate that BHR in asthma can be split into a relatively persistent component and a superimposed and more episodic component; best assessed respectively by direct and indirect stimuli [8]. Direct stimuli are seen as more sensitive and less specific than indirect challenges in relation to asthma, whereas indirect BHR is more responsive to treatment with inhaled corticosteroids and probably also more closely linked to airway inflammation [9].

BHR is reported from most [10–22] but not all [23–26] follow-up studies after preterm birth. Links between BHR and markers of eosinophil airway inflammation have been difficult to establish in ex-preterms [3,14]. However, recent data suggest presence of a neutrophilic airway inflammation in BPD, possibly reflecting mechanisms resembling those underlying chronic obstructive pulmonary disease (COPD) [27].

Mechanisms underlying increased BHR in preterm born survivors

BHR after preterm birth is poorly understood, and there is much debate as to what extent it is a structural disorder or reflects an active disease with on-going inflammation.

The 'structural disease scenario' can be seen as interstitial consequences of the acute phases of IRDS and BPD. Limitation of maximal expiratory airflow depends on airway resistance, which must reflect the cross-sectional dimension of medium sized bronchi, which depends on the internal elastic recoil of the lung, which again depends on adequate deposition of fibrous and elastic fibers in the pulmonary interstitium [28]. As evidence of airway obstruction is reported from virtually all studies of ex-preterm individuals, interactions between these factors must be negatively influenced by preterm birth. In fact, altered elastic and fibrous networks are described in lungs after exposure to oxygen supplementation and positive pressure ventilation [29]. Regarding BHR, the capacity of airway smooth muscle to shorten (and thereby reduce the size of the airway lumen) in response to a maximal cholinergic stimulus is attenuated by the elastic load (counter-pressure) that the lung parenchyma can impose on the airway smooth muscle when it contracts [30]. Loss of elastic recoil may thus lead to increased responsiveness to methacholine. This line of thinking supports the notion that BHR after premature birth is not an expression of airway mucosal inflammation, but of pulmonary parenchymal structural sequelae, and possibly related to distorted elastic and/or fibrous architecture of the interstitium.

Support for the 'active disease scenario' would require data that link BHR in ex-preterms to markers of airway inflammation. To date, only a few studies have investigated airway inflammation in the context of airway obstruction or BHR, and none have reported the eosinophilic pattern typical for childhood asthma.

As regards other types of inflammatory mechanisms, evidence of oxidative stress in the airways assessed from analyses of exhaled breath condensate led the authors to hypothesize that pro-inflammatory activity might be involved [31,32]. These findings need to be replicated by other research groups, but most agree that the airway disease that follows preterm birth should be seen as a distinct process to childhood asthma [33]. More research is clearly needed in this area. The question to what extent this respiratory disorder is due to stabilized early airway injuries and/or disrupted lung growth and development, or reflects an active ongoing disease, is currently unclear.

Agents used to evaluate direct BHR

Direct stimuli act directly on receptors located on the airway smooth muscle and may thereby induce airflow obstruction depending on the dose administered and the characteristics of the airways. Direct stimuli include methacholine and other muscarinic analogues, histamine, leukotrienes and prostaglandins. Methacholine is the most commonly used test substance. It acts directly on the muscarinic acetylcholine receptor, subtype M3 (mAChRsM3), located on the smooth airway muscle where it promotes contraction and thereby airway obstruction [34]. Methacholine BHR is a sensitive marker of asthma but not very specific [9,35]. Histamine works like methacholine through the cholinergic pathway; however, additionally also through irritant receptors like histamine H1 and H2 receptors and by activating sensory nerve fibers in the airways. The mechanisms involved in bronchial responsiveness to histamine is less well characterized and the relative contribution from direct versus indirect effects is also not clearly defined [36,37].

Direct airway responsiveness reflects airway smooth muscle function, caliber and structure more than it reflects airway inflammation, contrasting indirect stimuli that reflect airway inflammation and not so much airway muscle function.

Outcome measures used to quantitate direct BHR

The outcome measure most commonly used to measure the airway response to direct BHR agents is the induced change in forced expiratory volume in 1 s (FEV_1). Most protocols define a positive response as a drop of 20% in FEV_1 compared to the baseline value, although some use a decrease of 10% as cut-off. The provocation dose [PD] or provocation concentration [PC] needed to induce this reduction in FEV_1 is often referred to as PD_{20} or PC_{20}. Other outcome parameters that have been used in children include changes in oxygen saturation by 5%, induction of wheezing, or changes of lung function parameters other than FEV_1.

Methods used to test indirect BHR

These methods work through induction of bronchoconstriction by activating one or more inflammatory pathways in the bronchial mucosa, and involve release of mediators from inflammatory cells, and/or of neuropeptides from sensory nerves. Indirect stimuli that cause inflammatory cell mediator release include exercise [38] and eucapnic voluntary hyperventilation [39,40], non-isotonic aerosols [41], adenosine monophosphate (AMP) [42,43] and mannitol [44]. The airway response to indirect stimuli correlates better than methacholine with eosinophilic airway inflammation [42,45,46]. Atopic individuals are more likely to have BHR to AMP than non-atopic individuals [47], and a mannitol challenge is often negative in asymptomatic individuals with BHR to methacholine [48]. Indirect BHR improves more than direct BHR after institution of anti-inflammatory treatment [45] (Table 1).

Exercise

Exercise is the most commonly used method to test indirect BHR. Exercise-induced bronchoconstriction (EIB) describes the acute bronchial narrowing that occurs as a result of exercise. EIB commonly occurs in asthma, but is also reported in individuals without an established asthma diagnosis [49]. The symptoms of EIB are variable and nonspecific; presence or absences of specific respiratory symptoms have very poor predictive value for objectively confirmed EIB [50]. Several studies indicate that subjects who are predisposed to EIB have increased levels of exhaled nitric oxide, leukotrienes, mast cell mediators and epithelial cell release into the airway lumen [51–53]. Although the events that trigger this syndrome are not fully understood, it is clear that inflammatory mediators are released into the airways from eosinophils and mast cells [49]. Populations at risk, such as children and subjects with pre-existing cardiovascular disease, diabetes or lung disease are also more sensitive to environmental exposures that have been proposed to contribute to EIB [49]. The underlying mechanisms of EIB are not fully understood. We know that inhaled corticosteroids (ICS) are effective in EIB; however, corticosteroids do not seem to be as protective in subjects who have EIB but no established diagnosis of asthma [49].

Adenosine monophosphate (AMP)

AMP is a potent bronchoconstrictor in asthmatic patients, causing non-osmotic mast cell mediator release, primarily of histamine. AMP may have additional actions on neural pathways as the airway response to AMP is partially attenuated by atropine and ipratropium bromide [54,55]. AMP seems to be closely associated with airway inflammation in patients with asthma, and BHR after AMP

Table 1
Comparison of direct and indirect tests for bronchial hyper-responsiveness.

	Direct		Indirect				
	Methacholine	Histamine	Exercise	Mannitol	EVH/IHCA	AMP	Hypertonic saline
Benefits	Standard methods	–	Natural	Portable	Exercise surrogate	Correlates well to eosinophils	Induces sputum
Drawbacks	TLC* inhalation reduces sensitivity	Cough/Flush	Expensive/ Severe reaction	Not approved in every country	Severe reaction	Not approved in every country	Uncomfortable/Cough
Sensitivity for asthma	High				Low		
Specificity for asthma	Low				High		
Mainly measures	Muscle function/Airway Calibre				Inflammation		
Dosage needed to induce BHR	Low				High		

* TLC = Total Lung Capacity.

challenge appears to respond more readily to ICS than BHR after methacholine challenge [56–58]. BHR after AMP also seems to be related to eosinophilic airway inflammation, and can be used as a noninvasive marker of airway wall inflammation in asthma [59].

Eucapnic voluntary hyperventilation and isocapnic hyperventilation (EVH/IHCA)

There are a number of surrogates for exercise in relation to inducing bronchoconstriction, including eucapnic voluntary hyperventilation (EVH) of dry air, inhalation of hyperosmolar aerosols of 4.5% saline or dry powder mannitol. However, none of these surrogate tests are completely sensitive or specific for EIB, although they are all capable of identifying hyper-responsiveness. Isocapnic hyperventilation with cold air (IHCA) is similar to EVH, aiming to increase the sensitivity of BHR by inhaling cold and dry air.

Hypertonic saline

Inhalation of hypertonic saline (4,5%) was developed to investigate the hypothesis that EIB was caused by a transient increase in osmolarity of the airway surface liquid as a consequence of humidifying large volumes of air during exercise [60]. The response patterns seem to correlate well with exercise and EVH, and the same mediators associated with exercise and EVH have been measured in response to hypertonic saline. However, the test is not commonly used.

Mannitol

The mannitol challenge test was a further development of the hypertonic saline challenge test [60]. Mannitol was selected because it was generally regarded as a safe and feasible molecule to be used in both adults and children. Mannitol had been shown to stimulate release of histamine from mast cells, which was enhanced in the presence of anti-IgE. Inhalation of dry powder mannitol correlates well with other indirect challenges, and shares many of the same characteristics of adenosine monophosphate challenge. A positive test is consistent with the presence of inflammatory cells such as eosinophils and mast cells as well as their mediators, and can therefore be used to confirm ongoing active asthma.

BHR in preterm-born children

Respiratory symptoms after preterm birth are in clinical settings regrettably often seen as reflections of asthma; however, the underlying pathophysiology suggests different causal mechanisms. Asthma is characterized by a bronchial hyper-responsiveness that is provoked by exposures to specific physical, chemical or pharmacological stimuli [61]. As described, this BHR can be quantified by determining the cumulative dose of various provocative agents that induce a defined decline in lung function, most commonly measured by FEV_1 [62]. Importantly, BHR is a feature that is also observed in various chronic lung diseases other than asthma, and occasionally also in asymptomatic subjects [63–66]. The inconsistent extent of responsiveness that is observed when subjects suffering from different respiratory diseases are exposed to different provocative agents, indicate that various causal pathways must be involved. Thus, the causal mechanisms that lead to BHR are complex, not fully understood and most probably heterogeneous and vary between diseases.

BHR in asthma has been extensively studied, but the association with parameters of airway inflammation is still a matter of debate [67] and the issue of "structural" versus inflammatory mechanisms is still highly relevant [68,69]. For example, we know that BHR increases with decreasing FEV_1, cigarette smoking and atopy, whereas a decrease is observed with increasing age [70,71]. BHR in preterm born survivors has been much less studied, and no studies have found associations with markers of the inflammatory airway response that is so characteristic of asthma. In fact, the finding that ex-preterms with BPD exhibit BHR to methacholine but not to AMP support that bronchial hyper-responsiveness in preterm born children may not be mediated by inflammatory factors [13]. A couple of studies have provided data that made the authors speculate if BHR to methacholine in preterm born children could be related to in utero modifications of the muscarinic M3 receptors due to adverse exposures during fetal life [3,72]. They found extensive BHR to methacholine in growth restricted extremely preterm born 11 year old children and in six year old children with faltering intrauterine growth measured using repeated ultrasound assessments. We know that several factors, such as nicotine and ethanol, can cause epigenetic changes and interfere with the development and function of M3 receptors in various organs of the offspring [73,74]. In animal models, a protein-restricted diet [72] and even maternal stress [75] have been linked to BHR in the offspring. Thus, one may hypothesize that dysregulation of the M3 receptor due to adverse antenatal influences in preterm born children may alter the response to methacholine and lead to airway disease.

BHR in preterm born subjects have been studied using different approaches in population samples that have varied as regards important characteristics such as gestational age at birth, birthweight, age at testing and the treatment era into which the participants were born. Moreover, not all studies have included control subjects and the results are diverse. Thus, the results are challenging to summarize. We describe here a sample of the most relevant studies, classified according to the provocation agent they used.

Methacholine

Direct provocation with methacholine is the most commonly used agent, and was used in 11 of the 20 studies we selected. Six studies reported significant increases in BHR in subjects born preterm with BPD/CLD [11–16], and two studies reported the same tendency, however not statistically significant [10,17]. Three studies did not find any significant BHR response in the preterm born participants; however, the gestational age at birth of those included were generally above 34 weeks [23–25]. Significantly increased BHR in preterm born individuals without BPD was reported only in studies of subjects who were born extremely preterm. The data is heterogeneous and the diversity seemed related to characteristics of the study populations, most evidently gestational age at birth and the presence or absence of BPD. Overall, the studies suggest that there is a higher risk of BHR in the more immature preterm born survivors and in those who had prolonged neonatal requirements for oxygen, and thus qualified for a neonatal diagnosis of BPD.

Exercise induced bronchoconstriction (EIB)

Even if exercise is the most commonly used indirect BHR provocation, there are only a few studies reporting on EIB in preterm born subjects. Of the three studies that we identified [20–22], one used treadmill running and two used cycle ergometer, and all concluded that preterm born participants had a higher prevalence of EIB than the control group. In one study, the EIB responded to salbutamol [21]. All of these studies included preterm born subjects with a GA < 32 weeks or birthweight under 1500 g.
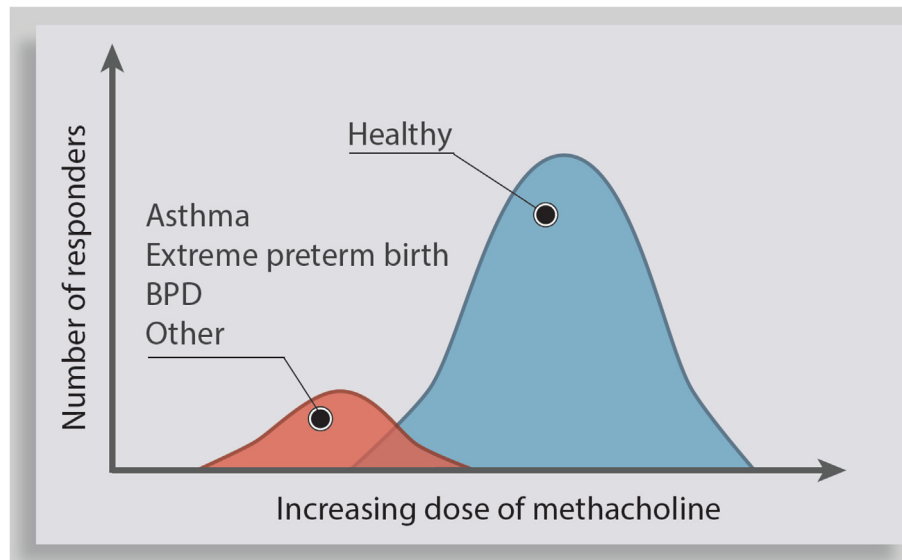
Fig. 2. Theoretical model illustrating the overlapping BHR response to methacholine in healthy and diseased. A responder is defined by a drop in FEV_1 of 20% or more to a given cumulative dose of methacholine. The bronchial responsiveness to methacholine demonstrated in preterm born individuals seems to overlap with that observed in asthmatic individuals.

Isocapnic hyperventilation

We identified one study that used isocapnic hyperventilation with cold air (IHCA). The authors reported that 25% of their preterm born population had a reduction in FEV_1 after IHCA exposure [76]. However, this study did not include a term born control group, and was performed in a context testing multiple preterm births; i.e. twins, triplets and quadruplets with gestational age at birth varying from 28 to 38 weeks. Given the 25% response rate, the conclusion proposed by the authors was that the prematurity and the intrauterine growth pattern caused by multiparity do not seem to be associated with BHR after provocation with IHCA.

Histamine

Histamine is classified as a direct provocation agent, and is the second most used substance (after methacholine) to test in preterm born subjects. The studies using histamine are heterogeneous, and the results inconsistent and difficult to interpret [26,77–79]; Two studies reported increased BHR in their preterm group [18,78], and the authors related their findings to very low birth weight or being born very preterm. One study from the Netherlands [26] looked at preterm born children (age range from 3 to 10 years) with and without respiratory symptoms in the neonatal period, and did not find any significant difference in BHR [26]. Koumbourlis et al. [77] longitudinally followed 17 preterm born children with CLD and found a gradual improvement in lung function into adolescence. At 15 years of age only 4 of 17 had BHR to histamine, and it appeared to be associated with small airway obstruction. They speculated if BHR is an inborn trait leading to both neonatal BPD and later lung disease, or if it is a result of neonatal airway injuries. Interpretation is, however, difficult as the authors did not include a comparison term-born group. The study by Bertrand et al. [79] compared preterm born subjects with and without RDS; each paired with a sibling born at term. BHR was also tested in their mothers. Expiratory flow was decreased in all the ex-preterm children, and this was strongly correlated to prolonged neonatal exposure to oxygen. BHR was elevated in all groups including their mothers and their term-born siblings, and the authors suggested that lung sequela in premature born children

without a history of RDS is related to BHR alone and that there also may be a relation between familial BHR and premature birth. This familial association for BHR has been reported also by others [80]; however, the Silverman group found that BHR after histamine where correlated to a history of asthma, but they were unable to find evidence to support the hypothesis that BHR in mothers has a causative role in the premature labor and subsequent BHR in their prematurely born children [81].

AMP

Two studies using AMP to address BHR in preterm born children reported no correlation with respiratory symptoms [12,13]. Kim et al. [13] used both methacholine and AMP, and found BHR to AMP only in those with symptoms of asthma. They also concluded that children with a neonatal history of BPD do not have the inflammatory airway response that is characteristic of asthma. Two previous studies support this notion, and stated that BHR to methacholine is present in both asthma and chronic lung diseases [82], whereas BHR to AMP seems to be present only in asthma [83] but not in CLD/BPD. This finding is consistent with a report from 2005 [14], also showing that BHR in preterm born subjects differed from asthma and was best explained by neonatal events, particularly prolonged requirements for oxygen treatment.

Conclusion

BHR seems to be increased in preterm born children, most evidently reported from studies that include the most immature participants or children with a neonatal history of bronchopulmonary dysplasia. Interesting differences seems to be present between BHR in children with asthma and lung disease after preterm birth, with no signs of eosinophilic inflammatory pathways reported for the latter group. This suggests that different pathophysiological mechanisms are involved in these two conditions, supporting the view that asthma therapy should not be uncritically applied in children with respiratory symptoms following preterm birth. BHR is an understudied characteristic in preterm born children, hampering a more fundamental understanding of their lung disorder and also complicating a research based approach to treatment.

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Directions for future research

- Investigate the causal factors that are involved in bronchial hyper- responsiveness in preterm born individuals, and particularly if it represents an active ongoing disease or a structural sequelae.
- Explore the life-long consequences of bronchial hyper-responsiveness after being prematurely born.
- Investigate links between bronchial hyper-responsiveness and clinical disease, and establish evidence based treatment schemes.

Acknowledgement

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