Upper and lower airway pathology in young children with allergic- and non-allergic rhinitis

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THE 3 ORIGINAL PAPERS ARE

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II. Chawes BL, Kreiner-Møller E, Bisgaard H. Upper and Lower Airway Patency are Associated in Young Children. Chest 2010 Jun;137(6):1332-7.
III. Chawes BL, Bønnelykke K, Kreiner-Møller E, Bisgaard H. Children with Allergic- and Non-Allergic Rhinitis have Similar Risk of Asthma. J Allergy Clin Immunol 2010 Sep;126(3):567-73.

GENERAL INTRODUCTION

ALLERGIC- AND NON-ALLERGIC RHINITIS IN CHILDHOOD

Definition and nomenclature

Rhinitis is an inflammatory disorder of the nasal mucosa characterized by nasal symptoms such as rhinorrhea, nasal obstruction, nasal itching and sneezing(1). The etiology of rhinitis is heterogeneous and the disease spectrum is typically classified as (1) infectious (acute or chronic); (2) allergic; (3) drug-induced (i.e. aspirin); (4) hormonal; (5) other causes (i.e. irritants); and (6) idiopathic rhinitis(2).

Allergic rhinitis is the most common form of non-infectious rhinitis(2). It is a hypersensitivity disorder of the nose where sensitized subjects produce specific immunoglobulin E antibodies (IgE) in response to allergens(3;4). Allergic rhinitis is defined by rhinitis symptoms and demonstrated IgE-mediated allergy (allergic sensitization) by positive skin prick test and/or elevated levels of serum allergen-specific IgE(2).

Non-allergic rhinitis is a disorder causing symptoms mimicking those of allergic rhinitis, but with no definite causal factor and without allergic sensitization(5). Non-allergic rhinitis is a diagnosis of exclusion and is defined by the absence of allergen-specific IgE and/or signs of infection and is also referred to as non-infectious non-allergic rhinitis(6).

Due to lack of adequate univocal phenotype categories in childhood and for practical purposes the majority of children with non-infectious rhinitis have been classified due to presence or absence of allergic sensitization into allergic- or non-allergic rhinitis(7). In this thesis, we used and refined that categorization of allergic- and non-allergic rhinitis.

Disease burden and prevalence

Allergic rhinitis is among the most common chronic disorders in childhood(8). The International Study of Asthma and Allergy in Childhood (ISAAC) has shown significant worldwide variations in disease prevalence varying from 1% to 15% in 6-7-year old children(9). The highest prevalence is found in westernized cultures where 10-15% of preschool children(10;11) and 15-20% of school-aged children are diagnosed with allergic rhinitis(12). The prevalence of allergic rhinitis as well as allergy and asthma has dramatically increased during the recent decades in industrialized countries in what has been called an “asthma and allergy epidemic”(13).

Although allergic rhinitis is usually not considered a severe disease nor is life threatening, the condition has a major impact on quality of life for the affected children(14;15). Sleep-disordered breathing and sleep apnoea causing disturbed sleep and daytime fatigue(16), impaired social activities because the child is less energetic, less happy and less peaceful(17), and a detrimental effect on school performance and difficulty in performing tasks in general have all been associated with childhood allergic rhinitis(18). Furthermore, because of the high prevalence in the general population, allergic rhinitis induces a considerable socio-economic impact due to health care utilization, treatment costs, and loss of work by affected families(19).

Studies of adults and adolescents with rhinitis have shown that the proportion of subjects with non-allergic rhinitis is at least 25%(20-23). The exact prevalence of non-allergic rhinitis in young children is unknown(6), but it has been observed in some birth cohorts that almost half of the children with rhinitis are without allergic sensitization(11;24).

There are no existing studies of the disease-specific impact on quality of life, activity impairment or sleep disturbance of childhood non-allergic rhinitis.

Diagnostic specificity, misclassification and under-diagnosis
Many studies use questionnaire-based diagnoses utilizing the ISAAC rhinitis core questions(25) to define allergic rhinitis as "a significant problem with sneezing, blocked or runny nose in the past 12 months in periods without accompanying cold or flu"(9). These questions have a relatively high positive predictive value of 70% for detecting allergic sensitization among children with symptoms of rhinitis, but are not helpful for detecting allergic rhinitis in a general population of children due to low sensitivity (26%)(26). This epidemiological approach may lead to misclassification and over-report of allergic rhinitis as it is difficult for the parents to separate a history of viral symptoms from non-viral symptoms(27).

Allergic rhinitis is often undiagnosed in clinical practice(27). This is probably partly because symptoms are trivialized or misinterpreted by parents who then fail to seek medical assistance and partly due to lack of attention from general practitioners. As an example, data from a study of 5-year-old children showed allergic rhinitis diagnosed by the general practitioners in 5% of the children compared to 10% when diagnosis was based on allergic sensitization and parent interviews in a research clinic(11).

Unfortunately, many studies of allergic- and non-allergic rhinitis are hampered by low diagnostic specificity due to misclassification, over- and under-diagnosing(27). This is a major problem because exact phenotyping is essential in order to study and improve our understanding of the nasal pathology behind such diagnosis. In addition, clear and univocal diagnoses are needed to perform proper studies of environmental and genetic risk factors.

UPPER AIRWAY PATHOLOGY IN CHILDREN WITH RHINITIS

Nasal patency
In the recent Pediatric Allergies in America survey, nasal blockage/congestion was found to be the primary symptom affecting children with rhinitis aged 4-17 years(17). Half of the 500 investigated children experienced nasal congestion on most of the affected days and 75% stated that this was the most bothersome symptom and that it was either moderately or severely bothersome. In parallel, 92% of the parents believed that nasal congestion was the most bothersome symptom for their children(17). There is evidence to suggest that nasal congestion is a key symptom in pediatric allergic rhinitis(2) and, therefore, rhinitis phenotyping may improve from objective measures of nasal airway patency.

Active anterior rhinomanometry has been applied in adults and adolescents with allergic rhinitis to evaluate nasal airway resistance and response to nasal decongestion after intranasal α-agonist (nasal decongestion test)(28-30). However, this technique is not applicable in young children for cooperative reasons(31), which is a particular problem as this age group has difficulties in interpretation and verbalization of nasal symptoms such as congestion/stuffiness(32).

Another more recent approach is acoustic rhinometry, which is a simple and non-invasive method for objective measurements of nasal airway patency(33), which has been validated against Computed Tomography(34) and Magnetic Resonance Imaging(35). The technique is well tolerated by both infants and preschool children(36-38) and provides assessment of the nasal cross-sectional area against distance as well as nasal volumes before and after decongestion(33). Reduced nasal airway patency and increased response to nasal decongestion have been shown in adults with allergic rhinitis(39), but there are no available reports on acoustic rhinometry measurements in young children with allergic- and non-allergic rhinitis.

Nasal inflammation
Allergic rhinitis is archetypically characterized by a T-helper 2 (Th2) cell deviated immune response involving a complex cascade of mediators such as interleukin-4 (IL-4), IL-5 and IL-13 which drive IgE production and recruitment of eosinophil granulocytes(40).

Upon contact with inhaled allergens an early-phase and subsequently a late-phase reaction is initiated(2). Within minutes, the early-phase reaction is promoted by degranulation of IgE-sensitized mast cells, which release both preformed and newly synthesized mediators such as cysteinyl leukotrienes, prostaglandins, histamine, and cytokines leading to the characteristic symptoms of allergic rhinitis(41). The late-phase reaction develops within hours to days and is characterized by influx to the nasal mucosa of inflammatory cells such as eosinophils, basophils, mast cells, neutrophils and mononuclear cells(42). The eosinophil granolucyte is the predominant inflammatory cell type in the chronic late-phase reaction and releases a series of proinflammatory mediators including cysteinyl leukotrienes, cationic proteins, eosinophil peroxidase, and major basic protein, which sustain nasal inflammation and symptoms such as nasal congestion(43).

Presence of nasal mucosal eosinophilic inflammation (nasal eosinophilia) is a hallmark of allergic rhinitis and has been associated with development, progression and severity of allergic rhinitis in children and teenagers(44;45). In particular, nasal eosinophilia correlates well with the symptom of nasal obstruction(46). In addition, some children with non-allergic rhinitis also have nasal eosinophilia termed non-allergic rhinitis with eosinophilia syndrome (NARES)(47). Although nasal scraping by Rhinoprobes® for assessment of nasal eosinophilia is simple to perform(42), there is a paucity of available data from preschool children with allergic- and non-allergic rhinitis.

Assessments of nasal mediator levels have previously been done in nasal lavage fluid collected after nasal allergen challenge(48-50). This is probably a poor reflection of their role during naturally occurring disease as allergen challenge exaggerates natural exposures. Furthermore, the nasal lavage fluid might dilute mediator levels below detection limit of the assays. Only recently we have contributed to the development of a novel Synthetic Absorptive Matrix (SAM) method, which enables detection of undiluted natural levels of nasal cytokines and chemokines(51). However, this method was not at our disposal for the studies of this thesis.

Objective assessments of allergic- and non-allergic rhinitis
In paper I, we studied the associations between rhinitis symptoms and objective measures of allergic sensitization, nasal airway patency end-points assessed by acoustic rhinometry, and nasal mucosal eosinophilic inflammation.

THE COEXISTENCE OF ASTHMA AND RHINITIS

The respiratory tract
The nose and lung share many features apart from their obvious anatomical connection including mucosal and immunologic similarities as well as functional complementarity(52;53). In particular, the nose conditions inhaled air by warming, humidifying, and filtering particles and gaseous materials in order to protect the lower airway homeostasis(53).

Asthma and rhinitis

Epidemiological studies of children, adolescents and adults from all parts of the world have consistently shown that asthma and rhinitis often coexist in the same patient(11;22;54-59). The majority of patients with allergic- and non-allergic asthma present rhinitis symptoms(2;56;59;60), whereas the prevalence of asthma among patients with rhinitis varies from 10% to 50%(54;55;57;59). Symptoms often predominate in one organ and are hidden or unrecognized in the other organ even though they exist. Under-diagnosis of concurrent asthma is common and is illustrated by a questionnaire survey of 12,000 subjects with allergic rhinitis showing that 30% without an asthma diagnosis might be considered to be asthmatics(61).

Allergic rhinitis is well-known to be associated with asthma constituting the "united airways concept" and stressed in the Allergic Rhinitis and its Impact on Asthma guidelines (ARIA)(2), but a growing amount of evidence now also support a link between non-allergic rhinitis and asthma(23;56;62;63). Studies among adolescents and adults with allergic- and non-allergic rhinitis have shown prevalence rates of concurrent asthma ranging from 33% to 51% in allergic rhinitis and from 9% to 39% in non-allergic rhinitis, respectively(20;22;23), indicating that asthma is more frequently associated with allergic rhinitis compared to non-allergic rhinitis in adolescents and adults. Data from pediatric populations are scarce, but one study of 5-year-old children reported coexisting asthma in 26% of children with allergic rhinitis versus 34% of children with non-allergic rhinitis(11), and another study of 10-year-olds showed a 34% prevalence of asthma in subjects with allergic rhinitis versus 25% in non-allergic rhinitis(21). However, the reported prevalence of concurrent asthma did not differ significantly among children with allergic- and non-allergic rhinitis in any of these studies(11;21).

Longitudinal data has established rhinitis, both allergic and non-allergic, as an important determinant of adult-onset asthma(64;65). These findings suggest a distinct temporal pattern in the development of upper and lower airway diseases and warrant increased awareness of children suffering from rhinitis. However, patients with an established diagnosis of both rhinitis and asthma also require special attention.

Children with coexisting rhinitis and asthma experience more asthma-related emergency room visits and hospitalizations(66;67), incur a higher asthma drug cost, and attend their primary physician more often than children with asthma alone(67). Recognition and proper treatment of rhinitis symptoms in patients with asthma are of utmost importance to ensure optimal quality of life(68) and to reduce potential life threatening asthma exacerbations(69).

Mechanisms behind nose-lung interactions

Both respiratory, neural and systemic pathways have been proposed to account for the common comorbidity of rhinitis and asthma(70). Loss of the protective functions of the nose is the simplest respiratory explanation(71), but altered nasal nitric oxide (NO) production may also contribute to the naso-bronchial cross-talk(72). Alternatively, nasal pharyngeal bronchial reflex mechanisms may act as the connecting link between nose and lung mediated by mechanical or chemical stimulation of a complex neural pathway including trigeminal and vagal nerves(73). However, the systemic pathway is currently the most widely accepted explanation for nose-lung interactions. Nasal allergen challenge has been shown to induce a systemic allergic response where absorption of inflammatory mediators (e.g. IL-5 and eotaxin) from sites of inflammation into the systemic circulation promotes release of eosinophils from the bone marrow, prolonged blood eosinophilia, and thereby theoretically systemic propagation of inflammation from nose to lung(74).

Little attention has been paid to the role of airway dimensions although congenitally small airway dimensions have been shown in early transient wheezers suggesting this may predispose to an increased wheeze propensity(75). Hitherto, no studies have addressed whether generalized diminished airway dimensions in the nose and lung could contribute to the communality between symptoms of rhinitis and asthma.

Upper and lower airway patency

In paper II, we examined whether upper and lower airway patency were associated assessing upper airway patency by acoustic rhinometry before and after nasal decongestant and lower airway patency by spirometry pre- and post bronchodilator. In addition, we investigated a possible association between upper airway patency and nasal eosinophilia, blood eosinophilia, and level of fractional exhaled nitric oxide (FeNO) as well as lower airway patency and nasal eosinophilia, blood eosinophilia, and FeNO.

BRONCHIAL INFLAMMATION IN ALLERGIC- AND NON-ALLERGIC RHINITIS

Sub-clinical bronchial inflammation

Nasal and bronchial inflammations are often related in the same patient even when symptoms only reveal as either rhinitis or asthma(76;77). Thus, bronchial inflammation can result from nasal allergen challenge in patients with allergic rhinitis perceiving nasal symptoms alone(76) and segmental bronchial provocation has been shown to induce nasal inflammation(77).

There is persuasive evidence supporting that subjects with allergic rhinitis without bronchial symptoms have impaired lung function including decreased forced expiratory volume in the first second (FEV1) and forced expiratory flow at 25–75% of the pulmonary volume (FEF25–75)(78-80). Increased prevalence of asymptomatic bronchial hyperresponsiveness to histamine or methacholine has also been demonstrated in adult rhinitics(55;79;81;82) and suggested in a few studies of children with allergic rhinitis(21;83;84). In addition, one study of adults with NARES without any history of respiratory symptoms showed bronchial hyperresponsiveness to methacholine in almost half of the patients(85). Together, these clinical data suggest a subclinical bronchial disease process in adults with rhinitis and emphasize rhinitis as a marker of a generalized airway disease. However, this issue has not yet been properly addressed in young children with allergic- and non-allergic rhinitis.

Allergic- versus non-allergic rhinitis

It is well established that asthma is a common comorbidity in both allergic- and non-allergic rhinitis(20;22;23). However, whether asthma associated with allergic- and non-allergic rhinitis represents different endotypes of asthma (contraction of endophenotype, i.e. subtype of disease associated with distinct clinical features(86)) apart from the obvious differential association with allergy, remains to be fully elucidated. Increased prevalence of airway hyperesponsiveness in subjects with allergic rhinitis compared to non-allergic rhinitis has been shown in school-aged children(21) and in a mixed population of adolescents and adults(22). Higher mean FeNO level has also been demonstrated

in adults with allergic- versus non-allergic rhinitis(23). These findings suggest different endotypes of asthma symptoms in the adults with allergic- and non-allergic rhinitis. This has not been studied previously in young children(6) which is important because atopic phenotypes shown distinct temporal patterns(87-89) and extrapolation of evidence beyond age-groups is probably not justifiable(90).

Asthma endotypes in children with allergic- and non-allergic rhinitis

In paper III, we investigated asthma endotypes associated with allergic- and non-allergic rhinitis in young children by comparing: (1) prevalence of asthma, eczema, food sensitization, and filaggrin null-mutations; (2) levels of total IgE, blood eosinophil count, and nasal eosinophilia; and (3) FeNO level, lung function, reversibility to β2-agonist, and bronchial responsiveness to cold dry air. Furthermore, we examined whether sub-clinical bronchial inflammation was present in children with allergic- and non-allergic rhinitis.

AIMS AND OBJECTIVES

The aim of this PhD thesis was to describe pathology in the upper and lower airways in young children with allergic- and non-allergic rhinitis. Such insight may increase our understanding of pathogenesis in general and might contribute to the discovery of new mechanisms involved in the communality between disorders of the upper and lower airways. This could direct future research in order to develop proper preventive measures as well as adequate monitoring and treatment of children with rhinitis.

THE SPECIFIC OBJECTIVES WERE

- To study nasal airway patency and nasal eosinophilia in children with investigator-diagnosed allergic- and non-allergic rhinitis.
- To study the association between upper and lower airway patency.
- To study asthma and intermediary asthma end-points in young children with allergic- and non-allergic rhinitis.

METHODOLOGY

DESIGN, SETTING AND PARTICIPANTS

COPSAC

All the studies in this thesis are based on data from the COpenhagen Prospective Studies on Asthma in Childhood (COPSAC).

COPSAC is an ongoing single-center prospective clinical birth cohort study of 411 children born to mothers with a history of asthma. During 1998-2001 the study enrolled all participating families from the region of greater Copenhagen, Denmark, excluding children born before 36 weeks of gestation and anyone suspected of chronic diseases or lung symptoms prior to inclusion. Recruitment, demographics, baseline characteristics and study design are described in details elsewhere(91-93).

Data quality

The children attended the clinical research unit one month after birth and subsequently every six months for scheduled clinical investigations according to standard operating procedures as well as for any acute symptoms from airways or skin during the first seven years of life. Doctors working in the clinical research unit evaluated symptoms of atopic disease from clinical examination supported by parents' daily diaries. Diagnosis and treatment followed predefined algorithms and were controlled by the doctors at the research clinic who acted as general practitioners for the cohort.

The prospective approach with close clinical follow-up and daily diary cards minimizes recall bias. Risk of misclassification and diagnostic variation is low as the families solely attended the doctors employed at the clinical research unit for diagnosis and treatment of any atopy related symptom rather than their family practitioner.

Comprehensive longitudinal objective assessments including blood sampling for measurement of atopic biomarkers, nasal scraping for nasal eosinophilia, acoustic rhinometry, FeNO, lung function assessed by spirometry and whole body plethysmography, bronchial reversibility and responsiveness are performed in accordance with internationally recognized guidelines(42;94-104). All measurements were done by highly trained research assistants at the COPSAC research unit following standard operating procedures. In addition, many measurements such as lung function, bronchial responsiveness to cold dry air and FeNO are obtained using computer-animated software with a specific children friendly approach.

Data validity and quality procedures follow "Good Clinical Practice". Symptom history is captured online during the visits to the COPSAC research unit and entered into a dedicated Oracle database on a novel SQL server. Objective measurements are double checked against source data and the database subsequently locked for further editing. An audit trail is run routinely.

Ethics

The COPSAC study is conducted in accordance with the Declaration of Helsinki and was approved by the Copenhagen Ethics Committee (KF 01-289/96 and KF 11-107/02) and the Danish Data Protection Agency (2008-41-1754). Informed consent was obtained from the parents at enrolment.

INVESTIGATOR-DIAGNOSED ALLERGIC- AND NON-ALLERGIC RHINITIS

Allergic sensitization

In this thesis, allergic sensitization is determined by measurement of serum specific IgE levels(96;104) using 0.35kU/L (radioallergosorbent test (RAST) class I) as cut-off to define a positive test.

Cumulative circulating levels of specific IgE antibodies against 8 common inhalant allergens (birch, timothy grass, mugwort, house dust mites, moulds, cat, dog and horse) were determined by ImmunoCAP assay(96;104;105) (Pharmacia Diagnostics AB, Uppsala, Sweden) in plasma collected at 6 years of age. Values of specific IgE ≥ 0.35kU/L were considered indicative of sensitization and were analyzed as a dichotomized measurement.

Allergic- and non-allergic rhinitis

Rhinitis was diagnosed by the COPSAC doctors based on parent interviews (not questionnaires) on rhinitis symptoms in the child's 6th year of life. The interview addressed rhinitis symptoms (sneezing, blocked nose, runny nose and nasal itching/rubbing), rhinitis medication usage (oral antihistamines, nasal steroid and/or nasal cromone trials), limitation of daily activities and sleep disturbance, eye involvement (itching/watery and red eyes), suspected precipitating factors, and time of year with symptoms. Based on these interviews, rhinitis was defined by persistent troublesome sneezing or blocked or runny nose in the past 12

months severely affecting the well-being of the child in periods without common cold or flu(26).

Allergic rhinitis was diagnosed in children with rhinitis and allergic sensitization to aeroallergens clearly related to the symptomatic periods (birch (April-May), grass (May-August), mugwort (July-August), moulds (May-October), house dust mites (October-February), and animals (when exposed)).

Non-allergic rhinitis was diagnosed in children with rhinitis without allergic sensitization to aeroallergens or without symptoms during periods of exposure to such allergens (clinically irrelevant sensitization).

Specific Methodologies of the Papers

Additional specific methodologies of this thesis are described in details together with the respective studies.

PAPER I

INTRODUCTION

In this paper, we studied nasal mucosal pathology in 6-year-old children with investigator-diagnosed allergic rhinitis, non-allergic rhinitis and healthy controls without allergic sensitization and symptoms of rhinitis. This was done by examining the associations between rhinitis symptoms and objective measures of allergic sensitization, nasal airway patency end-points assessed by acoustic rhinometry, and nasal mucosal eosinophilic inflammation.

We hypothesized that childhood allergic- and non-allergic rhinitis are of different pathologies. Evidence in favor of this hypothesis would be objective differences in nasal airway patency and nasal mucosal eosinophilic inflammation among children with allergic- and non-allergic rhinitis.

METHODS

Rhinitis diagnosis

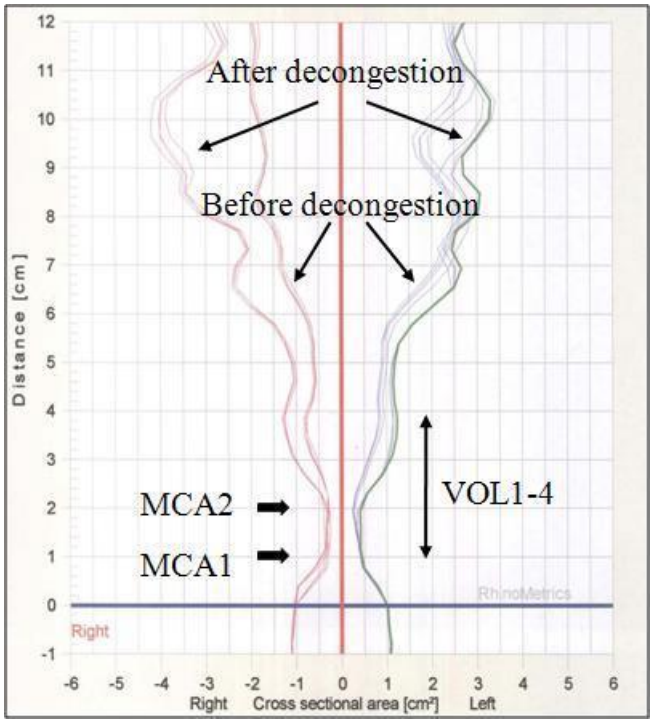
Diagnosis of allergic- and non-allergic rhinitis is described in the Methodology section. Nasal steroid usage was defined as intranasal steroids applied within the month prior to acoustic rhinometry and nasal scraping.

Nasal airway patency

Nasal airway patency was assessed by acoustic rhinometry performed twice in the child's 6th year of life both in and out of the pollen season. Measurements were made by trained research assistants at the COPSAC clinical research unit using the SRE 2100 continuous wide-band acoustic rhinometer with a small-sized adult anatomical nose adapter (RhinoMetrics, Interacoustics AS, Assens, Denmark). The subject was seated facing the examiner and stopped breathing for about 5 seconds with the probe tube applied to the nostril. Three independent measurements with a standard deviation less than 5% were obtained from each nostril before and 15 minutes after decongestion with one puff of intranasal xylomethazoline 1mg/ml.

The SRE 2100 continuous wide-band acoustic rhinometer provides cross-sectional area against distance curves before and after decongestion from topical α-agonist for each child. Minimum cross-sectional area 0-2.2 cm into the nasal cavity (i.e. internal isthmus) (MCA1) and 2.2-5.4 cm into the nasal cavity (i.e. anterior part of the inferior turbinate) (MCA2) are given automatically by the computer software (Figure 1).

FIGURE 1:



Acoustic rhinometry measurement before and after decongestion with intranasal α-agonist. The inner curves show measurements before decongestion, the outer curves measurements after decongestion. The vertical arrow represents the part of the area-distance curve which is integrated as the nasal volume 1-4 cm into the nasal cavity. MCA1 and MCA2 represent the minimum cross-sectional area at 0-2.2 cm from nares and 2.2-5.4 cm from nares, respectively.

A recent study showed that nasal resistance correlated better with changes in nasal volume compared to cross-sectional areas(38). Therefore, nasal volume 1-4 cm into the nasal cavity was calculated by integration of cross-sectional area against distance curves as suggested by previous studies(38;106;107).

Selection of nasal airway patency end-points was based on coefficients of variation of the change in acoustic rhinometry variables after decongestion. Coefficients for MCA1, MCA2, and nasal volume from 1-4 cm (VOL1-4) were calculated as:

$$\frac{\textbf{Mean difference (variable after decongestion} - \textbf{variable before decongestion)}}{\textbf{standard deviation difference}}$$

Nasal volume 1-4 cm into the nasal cavity yielded the highest coefficient of variation, i.e. improved signal to noise ratio, and was, therefore, chosen for further analysis.

The nasal airway patency end-points for association analysis were

- Baseline nasal airway patency: Absolute nasal volume 1-4 cm into the nasal cavity.
- Decongested nasal airway patency: Absolute decongested nasal volume 1-4 cm into the nasal cavity.
- Nasal responsiveness from topical α-agonist calculated as:

$$\frac{(\textit{Decongested nasal airway patency} - \textit{baseline nasal airway patency})}{\textit{baseline nasal airway patency}} \times 100\%$$

We selected the lowest values from the 2 observations to adjust for seasonal variation due to pollen season. The variables were analyzed categorized in quartiles.

Nasal eosinophilia

Nasal eosinophilia was assessed by nasal scraping performed twice in the child's 6th year of life both in and out of the pollen season. Nasal mucosal specimens were obtained by gently scraping the anterior part of the inferior turbinate with Rhinoprobes® (Arlington Scientific Inc, Arlington, TX). The specimens were transferred onto glass slides, air-dried for 30 minutes, fixed in 95% ethyl alcohol for 3 minutes and stained by May-Grünwald-Giemsa method. Eosinophils were counted by light microscopy at high-power (oil immersion, x1000) by two experienced cytologists, blinded to the rhinitis diagnosis. Rating was done according to Meltzer's semi-quantitative scale evaluating the mean number of eosinophils per 10 high-power field: (0) 0 cells, (½+) 0.1-1.0 cells, (1+) 1.1-5.0 cells, (2+) 5.1-15.0 cells, (3+) 15.1-20.0 cells, (4+) >20.0 cells(42). Specimens without respiratory epithelium or specimens with less than 10 high-power fields were excluded. Nasal eosinophilia was defined as ≥ 1+ and analyzed as a dichotomized variable. The subjects were judged to have eosinophilic inflammation if any one of the two specimens showed nasal eosinophilia.

Statistics

Inter- and intraobserver variations of the two cytologists were analyzed by weighted kappa values.

The associations between allergic rhinitis, non-allergic rhinitis, acoustic rhinometry variables and nasal eosinophilia were analyzed by graphical models, distinguishing first between direct, indirect, and spurious relationships, and secondly adjusting for confounding and effect modification of direct relationships by estimation of conditional relationships. Chi-square statistics and partial gamma coefficients were used during the analysis. All p values reported are Monte Carlo approximations of exact conditional p values; a p value ≤ 0.05 was considered significant.

Graphical models(108) are multivariate statistical models applied to analyze high-dimensional contingency tables and are employed here to reveal complex interactions between several out-comes. A graphical model is defined by a Markov graph where variables are presented as nodes, and significant associations between variables by lines. Associations between out-comes are described by gamma correlation coefficients and p values by Monte Carlo approximations(109).

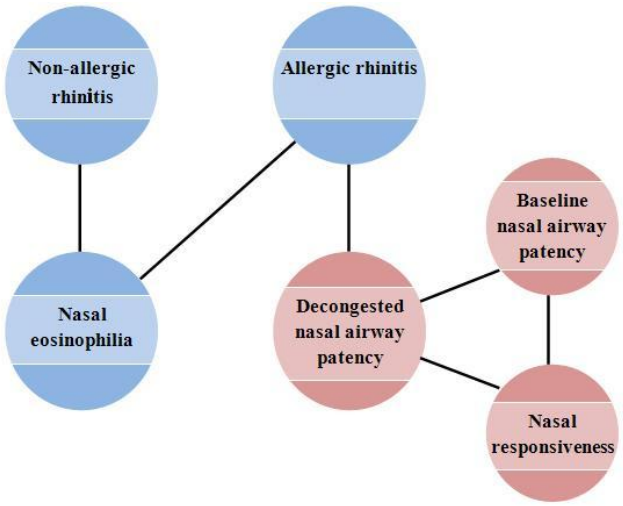
MAIN RESULTS

Baseline characteristics

By age 6 years, we completed a doctor interview on rhinitis symptoms and measurements of serum specific IgE, nasal eosinophilia, and acoustic rhinometry in 255 of the cohort of 411 infants. The study group was characterized by significantly higher prevalence of eczema and wheeze in the first 18 months of life and higher income compared to subjects without follow-up on these end-points, whereas there were no significant differences regarding sex, siblings and family history of allergic rhinitis (data not shown).

Rhinitis was diagnosed in 83 children (33%) and allergic sensitization against inhaled allergens in 66 children (26%). Allergic rhinitis could be defined in 23 children (9%) and non-allergic rhinitis in 60 children (24%); 34 children (13%) had asymptomatic sensitization. The control group without rhinitis diagnosis or allergic sensitization consisted of 138 children (54%).

FIGURE 2:



Graphical model (modified Markov graph) showing the relationships between allergic rhinitis, non-allergic rhinitis, nasal eosinophilia, baseline nasal airway patency, decongested nasal airway patency and nasal responsiveness.
The model investigates all potential associations between all variables (nodes) in the model. Significant associations between variables are presented as lines; no line between two nodes indicates a non-significant relationship.
The model is corrected for sex, height and nasal steroid usage.

Within the allergic rhinitis group, 52% were sensitizised against birch; timothy grass 78%, mugwort 17%, house dust mites 35%, moulds 26%, cat 35%, dog 35% and horse 22%. Thirty percentages were sensitizised to 1 inhaled allergen; 17% to 2; 22% to 3; 4% to 4; 9% to 5; 9% to 6 and 9% to 7 inhaled allergens.

Nasal eosinophilia was found in 18 children: 5 controls, 6 allergic rhinitis subjects, and 7 non-allergic rhinitis subjects.

Interobserver variation of the two cytologists was 0.9; intraobserver variations were 0.95 and 0.93, respectively.

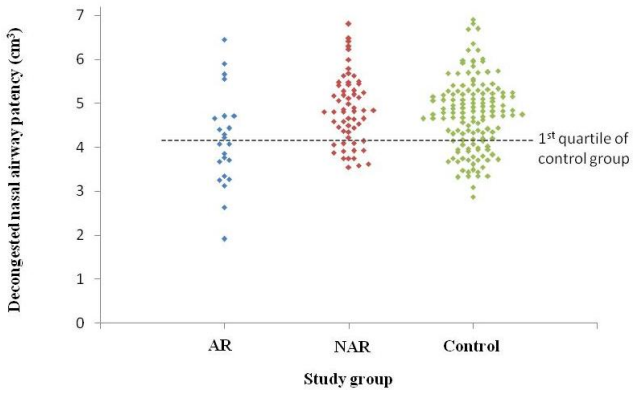
End-point associations

Figure 2 illustrates the associations between allergic rhinitis, non-allergic rhinitis, nasal eosinophilia and baseline nasal airway patency, decongested nasal airway patency as well as nasal responsiveness in a multivariate graphical model adjusted for sex, height and nasal steroid usage. Decongested nasal airway patency was significantly associated with a diagnosis of allergic rhinitis (p=0.004), but not with a diagnosis of non-allergic rhinitis (p=0.29). Neither baseline nasal airway patency nor nasal responsiveness were directly associated with allergic- or non-allergic rhinitis.

Figure 3 shows that 52% (12/23) of the allergic rhinitis subjects and 23% (14/60) of the non-allergic rhinitis subjects had decongested nasal airway patency below the 1st quartile of the healthy controls. This suggests that subjects with allergic rhinitis have reduced decongested nasal airway patency, i.e. irreversible nasal airway obstruction, whilst non-allergic rhinitis subjects have decongested nasal airway patency largely similar to healthy controls.

Nasal eosinophilia was directly and significantly associated with both allergic rhinitis (p=0.000) and non-allergic rhinitis (p=0.014) (Figure 2). Nasal eosinophilia was found in 26% (6/23) of subjects with allergic rhinitis and 12% (7/60) of subjects with

FIGURE 3:



Decongested nasal airway patency in the study group. The first quartile of the healthy controls (no rhinitis symptoms and no allergic sensitization) is shown as a dashed horizontal line.
AR=Allergic Rhinitis; NAR=Non-Allergic Rhinitis.

non-allergic rhinitis compared to 4% (5/138) in subjects with neither, suggesting that nasal eosinophilia is more closely associated with allergic rhinitis than non-allergic rhinitis.

Associations between rhinitis diagnosis and abnormal nasal findings

The relationships between nasal eosinophilia, irreversible nasal airway obstruction and allergic rhinitis and non-allergic rhinitis are illustrated by the Venn diagrams in Figure 4. The overlapping and non-overlapping areas between rhinitis diagnosis and abnormal nasal findings describe sensitivity and specificity of the objective measures. The figures illustrate that irreversible nasal airway obstruction is closer associated (greater overlap) with allergic rhinitis than with non-allergic rhinitis. Still, 39% (9/23) of subjects with allergic rhinitis presented neither nasal eosinophilia nor irreversible nasal airway obstruction as compared to 68% (41/60) of subjects with non-allergic rhinitis. Conversely, 28% (5/18) of subjects with nasal eosinophilia and 63% (45/71) of subjects with irreversible nasal airway obstruction had neither allergic nor non-allergic rhinitis.

DISCUSSION

Principle findings

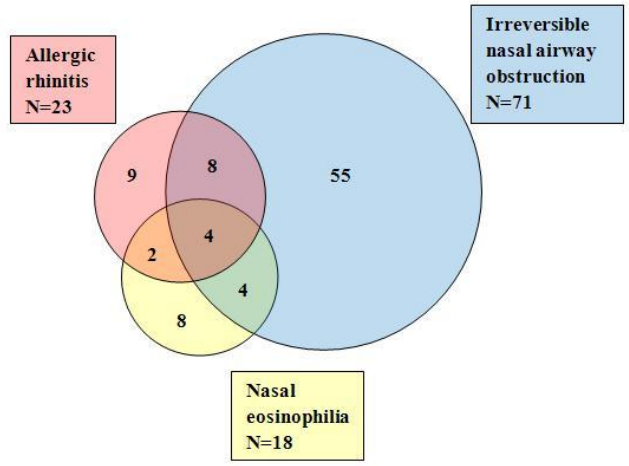
This is the first comparative study of nasal airway patency and nasal eosinophilia in young children with allergic- and non-allergic rhinitis.

Nasal mucosal eosinophilic inflammation was more closely associated with allergic rhinitis than with non-allergic rhinitis in 6-year-old children suggesting a stronger association between upper airway inflammation and allergic rhinitis compared to non-allergic rhinitis.

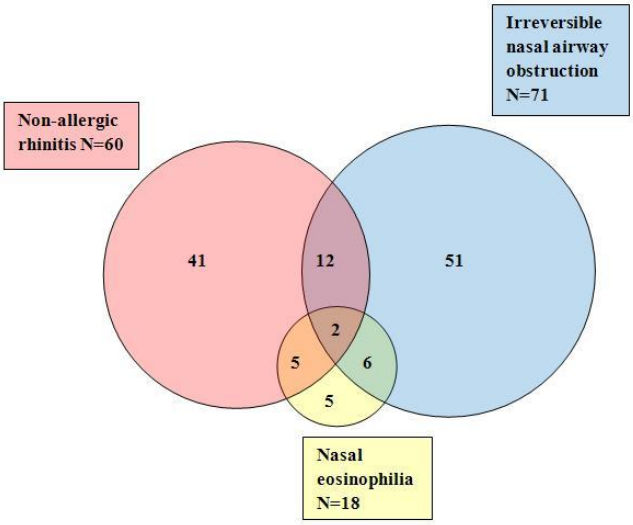
Irreversible nasal airway obstruction was strongly associated with allergic rhinitis in 6-year-old children, whilst there was no such association with non-allergic rhinitis. This suggests that chronic inflammation and structural remodeling of the nasal mucosa are part of allergic rhinitis, but not non-allergic rhinitis in children at 6 years of age.

FIGURE 4:

A)



B)



Venn diagrams showing the relationship between nasal eosinophilia, irreversible nasal airway obstruction, and allergic rhinitis (A) and non-allergic rhinitis (B). Irreversible nasal airway obstruction is defined as decongested nasal airway patency below the 1st quartile of the healthy controls.

Other studies

During the recent 4 decades, nasal mucosal eosinophilia has consistently been associated with a history as well as severity of allergic rhinitis in children aged 2-15 years(44;45;110-118). However, although many studies report high specificity of nasal eosinophilia for the diagnosis of allergic rhinitis (93% to 100%(45;112;113;116)), the clinical significance of this contribution is questioned by low sensitivity (14% to 69%(45;112;113;116)). Comparison of studies is significantly hampered by different sampling procedures including nasal blowing, nasal swabbing, nasal scraping and nasal lavage techniques as well as different staining methods and differences in quantification/semi-quantification of nasal eosinophilia. In addition, rhinitis phenotyping is not consistent from study to study and many studies include children in broad age ranges (e.g. 2-12 year old(44)) and investigate mixed populations of children and adults(111). All these issues may partly explain why the preva-

lence of nasal eosinophilia ranges tremendously from 10% to 69%(45;111-113;116;119). Despite the ambiguous usage of nasal eosinophilia in general clinical practice, nasal eosinophilia is highly correlated to immunological parameters and inflammation in allergic rhinitis(46) making it valuable for studies of upper airway pathology.

There are numerous studies of infants(34;36;106), preschool-aged children(34;37;38;106;107;120;121), and school-aged children(37;107;120;122) which report on the feasibility of acoustic rhinometry measurements and/or aim to establish reference material in different age groups. A few studies including a nasal cavity model(38) and mixed populations of children and adults(122) compared different acoustic rhinometry end-points such as minimum cross-sectional areas and nasal volumes before and after nasal decongestion. In accordance with our findings, these studies consistently found nasal volume to be the most sensitive measure of change in nasal airway patency(38;122).

In children, the primary clinical application of acoustic rhinometry has been in oto-rhino-laryngology for evaluation of adenoid size and the effect of different surgical procedures(123;124). In adults, applications have been in nasal decongestion test and evaluation of nasal airway patency after nasal allergen challenge techniques, and differences between normal and allergic rhinitis subjects have been described(39;125;126). Only one previous childhood study of children aged 4-13 years has evaluated the relationship between rhinitis and acoustic rhinometry variables(107) finding no association between baseline nasal airway patency (MCA1, MCA2, VOL0-4, VOL1-4, VOL2-5) and current rhinitis, which is in contrast to our findings. However, the rhinitis diagnosis did not contain allergy status, i.e. the subjects were a mixture of allergic rhinitis and non-allergic rhinitis. Furthermore, the children were not evaluated after nasal decongestion. No previous study has compared nasal airway patency in young children with allergic- and non-allergic rhinitis.

Meaning of the study

Allergic rhinitis was significantly associated with nasal mucosal eosinophilia, which has been related to sustained nasal obstruction in allergic rhinitis subjects(46) and may lead to structural remodeling with thickening of the reticular basement membrane; a phenomenon that may exist to a greater extent than previously thought in allergic rhinitis subjects(127). Allergic rhinitis was also significantly associated with irreversible nasal airway obstruction suggesting chronic inflammation and structural remodeling of the nasal mucosa in children at the age of 6 years. In agreement with this, a recent study in adults with allergic rhinitis showed that increased duration of allergic rhinitis is associated with an impaired response to nasal decongestion(128).

Chronic inflammation and structural remodeling of the upper airways might be part of a generalized remodeling of the airways including the lower airways(70). Loss of the protective functions of the nose is the simplest mechanistic explanation while absorption of inflammatory mediators (e.g. IL-5 and eotaxin) from sites of inflammation into the systemic circulation has been shown to result in release of eosinophils from the bone marrow, prolonged blood eosinophilia, and thereby possibly systemic propagation of disease from nose to lung(74).

Children with non-allergic rhinitis exhibited no change in the nasal airway patency, but some nasal mucosal eosinophilia albeit less than children with allergic rhinitis. This association may reflect local nasal IgE production ("entopy") in a fraction of non-sensitized rhinitis subjects, who over time will turn allergic(129) or eosinophilic inflammation triggered by other mechanisms than allergy.

The objective measures of nasal mucosal eosinophilia and nasal airway patency provide important insight into the pathology associated with allergic- and non-allergic rhinitis. However, they are not sensitive as 39% of children with allergic rhinitis presented neither nasal eosinophilia nor abnormal decongested nasal airway patency. This could be explained by short duration of allergic rhinitis since increased duration of allergic rhinitis is associated with an impaired response to nasal decongestion(128). Likewise, 68% of children with non-allergic rhinitis presented neither nasal eosinophilia nor abnormal decongested nasal airway patency suggesting that chronic inflammation and structural remodeling is not part of non-allergic rhinitis. A complementary and possible reason for the poor sensitivity of the methods is the issue of misclassification.

The low number of allergic rhinitis children with eosinophilic inflammation (26%) stresses that nasal eosinophilia is not useful in clinical practice with children at age 6.

Strengths and limitations

The major strength of this study is the standardized objective assessments. Nasal mucosal eosinophilia was evaluated from strict criteria with high agreement among the two cytologists. Nasal airway patency was assessed objectively by acoustic rhinometry before and after decongestion from topical α-agonist.

It is also a major strength that the diagnosis was made solely by the doctors at the COPSAC clinical research unit based on parent interviews and not on questionnaires. The diagnosis was in principle based on the traditional definition of "a significant problem with sneezing, blocked or runny nose in the past 12 months in periods without accompanying cold or flu"(9), but the clinical interviews by trained doctors at the single research unit allowed in-depth validation of the history. Furthermore, 77% of mothers in this high-risk cohort had a diagnosis of allergic rhinitis which improves symptom recognition. Additionally, allergic sensitization was evaluated as relevant versus irrelevant based upon congruence between exposure and symptoms.

We found a 9% prevalence of allergic rhinitis in our high-risk population all born to mothers with a history of asthma. Misclassification of rhinitis in children is common(11;26); it is probably under-diagnosed in clinical practice while questionnaire based surveys may over-report the prevalence(26;27). A recent study reported allergic rhinitis diagnosed by their general practitioner in 5% of 5-year-old children compared to 10% when diagnosis was based on sensitization and parent interviews in a research clinic(11). Another unselected cohort reported 15% of 7-year-old children were diagnosed with allergic rhinitis based on questionnaire and specific IgE against birch and grass(10).

The external validity of the study is limited from the setting of a high-risk cohort and for this reason the acoustic rhinometry reference values in the control group might differ from the background population. Furthermore, the study group displayed more eczema and wheeze as compared with the drop-out group and possibly an increased risk of eosinophilic inflammation and abnormal nasal airway patency.

The internal validity, however, is not affected by the high-risk nature of the cohort and the associations between rhinitis symptoms, allergic sensitization, nasal eosinophilia and nasal airway

patency are probably unaffected from the increased risk of atopy in the cohort.

CONCLUSIONS AND PERSPECTIVES

Allergic- and non-allergic rhinitis are of different pathologies as suggested from their different association with irreversible nasal airway obstruction and nasal eosinophilic inflammation. Children with allergic rhinitis by age 6 years are characterized by nasal mucosal eosinophilia and irreversible nasal airway obstruction suggesting chronic inflammation and structural remodeling of the nasal mucosa contrasting non-allergic rhinitis with less indication of chronic inflammation.

Future research

Chronic inflammation and structural remodeling of the nasal mucosa in allergic rhinitis children already at age 6 years might have important implications for rhinitis management in terms of pharmacological treatment and allergen avoidance. Allergic rhinitis in young children is presumably undertreated and indications of chronic inflammation should call for future studies addressing whether early intervention controls disease propagation.

The possible mechanisms driving chronic upper airway inflammation should also be investigated to seek new pathways for prevention and treatment. The allergic- and non-allergic rhinitis disease spectrum probably consists of several endotypes of rhinitis with distinct temporal patterns, clinical features, and underlying molecular mechanisms requiring customized treatment and guidance. Rhinitis endotyping could improve from studies utilizing the Synthetic Absorptive Matrix(51) method to characterize the complex naturally occurring nasal cytokine and chemokine levels and interactions as well as from studies describing the differential nasal immunological response to pharmaco- and immune therapy.

Genotype-(endo)phenotype association studies using objective nasal end-points such as irreversible nasal airway obstruction and nasal eosinophilia are also of great interest. Studying intermediate phenotypes of diseases in early life instead of the diseases themselves might give clues for new potentially modifiable pathways. Apart from genetic analysis, studies of environmental, intrauterine or early life risk factors driving these intermediate phenotypes or endotypes of rhinitis, may lead to new insight as there is increasing evidence suggesting that joint genetic and environmental factors underlie the developmental origins of health and disease (the DOHaD concept).

PAPER II

INTRODUCTION

In this paper, we utilized the nasal airway patency end-points derived from paper I to objectivize a possible linkage between the upper and lower airways in both healthy and atopic children.

Data from paper I suggested that decongested nasal airway patency is a sensitive measure of nasal inflammation(130). In addition, it is a general belief that bronchial reversibility to the β2-agonist and post-β2 FEV1 reflects bronchial inflammation(131-133). Therefore, an association between upper and lower airway patency would support the concept of a continuous nasobronchial airway inflammation process.

The aim of the current study was to examine whether upper and lower airway patency were associated. We assessed upper airway patency by acoustic rhinometry before and after topical α-adrenergic treatment and lower airway patency by spirometry before and after inhaled β2-agonist.

Subsequently, we investigated the association between upper airway patency and nasal eosinophilia, blood eosinophilia, and FeNO as well as the association between lower airway patency and nasal eosinophilia, blood eosinophilia, and FeNO.

METHODS

Diagnoses of allergic rhinitis, non-allergic rhinitis and allergic sensitization are described in the Methodology section. Definition of nasal steroid usage as well as assessment of nasal airway patency and nasal eosinophilia is described under paper I.

Upper airway patency

Decongested nasal volume 1-4 cm into the nasal cavity was selected as primary end-point as a measure of irreversible upper airway obstruction based on our findings in paper I(130). Baseline nasal volume 1-4 cm into the nasal cavity was used as secondary end-point.

Lower airway patency

Maximum FEV1 was assessed by spirometry from up to five technically acceptable maneuvers in accordance with international criteria for reproducibility(94) using the MasterScope system 754916 spirometer (Erich Jäeger, Würtzburg, Germany). Spirometry was performed in the child's 6th year of life, at baseline and 15 minutes after use of an inhaled β2-agonist (two puffs of terbutaline 0.25mg/dose in a pressurized metered dose inhaler with a spacer). All measurements were obtained with computer-animated volume driven incentive well-known to the children.

Post-β2 FEV1 was chosen as primary end-point as a measure of irreversible lower airway obstruction. Baseline FEV1 was used as secondary end-point.

Atopic biomarkers

FeNO was assessed by an online technique(99) using NIOX FLEX (Aerocrine, Solna, Sweden). Blood was sampled at age 6 years for measurement of eosinophil count (109/L) and total IgE. The level of total IgE was determined by ImmunoCAP (Phadia)(96) with a detection limit of 2kU/L.

Anthropometry

Height, weight and head circumference were measured the same day as acoustic rhinometry and spirometry assessments.

Asthma diagnosis

Current asthma during the 6th year of life was diagnosed according to the GINA guidelines as previously detailed(93), based on respiratory symptom diaries filled in on a daily basis by the parents; symptoms judged by the doctors at the clinical research unit to be typical of asthma (e.g. exercise induced symptoms, prolonged nocturnal cough, recurrent cough outside common cold, symptoms causing wakening at night); in need of intermittent rescue use of inhaled β2-agonist; responding to a 3-month course of inhaled corticosteroids and relapsing when stopping treatment.

Statistics

Associations between upper and lower airway patency were studied using generalized linear models with decongested nasal volume 1-4 cm into the nasal cavity as continuous outcome varia-

ble and post-β2 FEV1 as continuous explanatory variable. We adjusted the models for sex, allergic sensitization, rhinitis, asthma, nasal and inhaled steroid usage, height, weight, head circumference, body mass index (BMI) and forced vital capacity (FVC) by adding the variables as covariates to the models.

Interactions between sex, allergic sensitization, rhinitis and asthma and the studied associations between upper and lower airway patency were tested by adding cross-products to the models.

Associations between upper airway patency and atopic biomarkers and between lower airway patency and atopic biomarkers were investigated using generalized linear models corrected for sex, nasal steroid usage and inhaled steroid usage.

Results are reported as β-coefficients with 95% CI, a p value ≤0.05 is considered significant. All analyses were made in SAS version 9.1 for Windows.

MAIN RESULTS

Baseline

We investigated 276 of the cohort of 411 infants by acoustic rhinometry and spirometry in their 6th year of life: 253 completed baseline FEV1 the same day as nasal airway patency and 221 had concomitant post-β2 FEV1 and decongested nasal airway patency.

The study group was characterized by an increased prevalence of wheeze during the first 18 months of life compared to children not included in this current study, whereas the prevalence of allergic sensitization against aeroallergens was equally distributed. The study group had higher income and more fathers with asthma, whereas there were no differences regarding sex, older siblings and family history of allergic rhinitis and allergic sensitization against aeroallergens (univariate tests, data not shown).

Allergic sensitization against inhaled allergens was found in 59 children (27%). Rhinitis was diagnosed in 74 children (33%): allergic rhinitis was diagnosed in 21 children (10%) and non-allergic rhinitis in 53 children (24%). Thirty-three children (15%) had current asthma by age 6. Study group characteristics and objective assessments are given in Table 1.

Upper and lower airway patency

Decongested nasal airway patency was significantly associated with post-β2 FEV1, p=0.007 (Figure 5). After adjusting the model for sex, height, weight, head circumference, BMI, FVC, allergic sensitization, rhinitis, asthma, nasal and inhaled steroid usage, the estimated change in decongested nasal airway patency per 1 litre increase in post-β2 FEV1 (β-coefficient) was 2.85cm3 (95% CI, 0.42 to 5.29; r2=0.16; p=0.02).

There was no evidence for interaction with sex (p=0.64), allergic sensitization (p=0.37), rhinitis (p=0.50) or asthma (p=0.83) and the association of upper and lower airway patency.

Figure 6 shows the three-dimensional relationship between decongested nasal airway patency, post-β2 FEV1 and height. The figure illustrates that the association between upper and lower airway patency is independent of height.

Baseline nasal airway patency was also significantly associated with baseline FEV1, p<0.001. After adjusting for sex, height, weight, head circumference, BMI, FVC, allergic sensitization, rhinitis, asthma, nasal steroid usage and inhaled steroid usage, the association remained significant (β-coefficient, 0.89cm3; 95% CI, 0.26 to 1.51; r2=0.18; p=0.01).

TABLE 1:

Characteristic	Study group (N=221)
Male (N and %)	103 (47%)
Age (mean and SD), yrs	5.3 (0.3)
Height (mean and SD), cm	113.91 (4.9)
Weight (mean and SD), kg	20.3 (2.8)
Head circumference (mean and SD), cm	51.9 (1.4)
Body Mass Index (mean and SD), kg/m^2	15.7 (1.3)
Allergic sensitization* (N and %)	59 (27%)
Allergic Rhinitis (N and %)	21 (10%)
Non-Allergic Rhinitis (N and %)	53 (24%)
Asthma† (N and %)	33 (15%)
Baseline nasal airway patency (mean and SD), cm^3	2.86 (0.66)
Decongested nasal airway patency (mean and SD), cm^3	4.70 (0.80)
Nasal reversibility to α-agonist (mean and SD), %‡	+68.7 (30.4)
FEV1 (mean and SD), L	1.16 (0.19)
Post-β2 FEV1 (mean and SD), L	1.17 (0.16)
Bronchial reversibility to β2-agonist (mean and SD), %§	+2.7 (11.8)
FeNO‖ (GM and 95% CI), ppb	7.3 (2.7-19.7)
Blood eosinophil count (GM and 95% CI), 10^9/L	0.33 (0.08-1.30)
Total IgE (GM and 95% CI), kU/L	44.8 (3.2-629.2)
Nasal eosinophilia (N and %)**	16 (8%)

Study group characteristics.

Definition of abbreviations: SD=Standard Deviation; FEV1=Forced Expiratory Volume in the first second; GM=Geometric Mean; CI=Confidence Interval; FeNO=Fractional Exhaled Nitric Oxide.

**Allergic sensitization is any sensitization (specific IgE ≥ 0.35kU/L) towards birch, timothy grass, mugwort, house dust mites, moulds, cat, dog and horse.*

†2 subjects with missing asthma status.

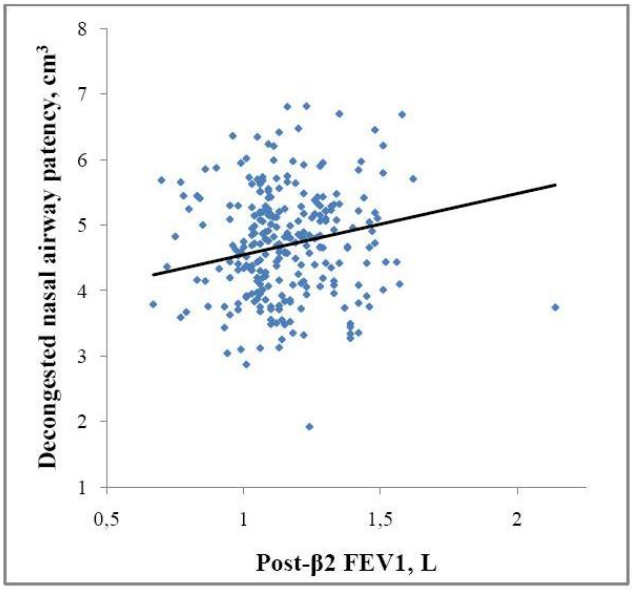
‡Nasal reversibility = ((Decongested nasal airway patency-Baseline nasal airway patency)/ Baseline nasal airway patency) x 100.

§Bronchial reversibility = ((Post-β2 FEV1-FEV1)/FEV1) x 100.

‖ 77 subjects with missing FeNO measurement.

***17 subjects with missing nasal scrape.*

FIGURE 5:



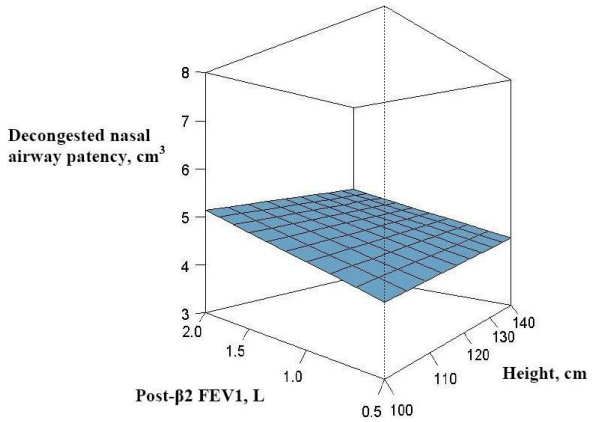
Association between decongested nasal airway patency and post-β2 FEV1.

There was no association between reversibility of the upper and lower airways (Table A1, Appendix A).

Upper and lower airway patency and atopic biomarkers

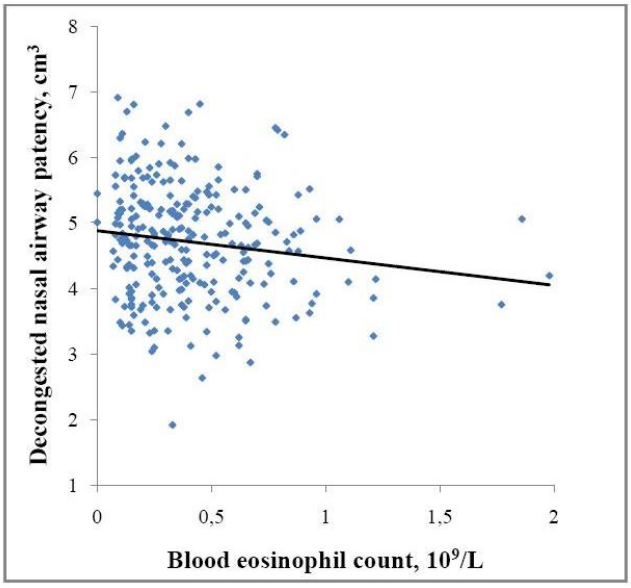
Blood eosinophil count was inversely associated with decongested nasal airway patency (Figure 7), whereas there was no

FIGURE 6:



Three-dimensional relationship between decongested nasal airway patency, post-β2 FEV1 and height.

FIGURE 7:



Inverse association between decongested nasal airway patency and blood eosinophil count.

such association with post-β2 FEV1. After adjusting the model for sex, nasal steroid usage and inhaled steroid usage, the estimated decrease in decongested nasal airway patency, i.e. increased nasal airway obstruction, per 1x109/L increase in eosinophil count was 0.42cm3 (β-coefficient, -0.42cm3; 95% CI, -0.77 to -0.07; r2=0.17; p=0.02).

In addition, nasal eosinophilia was inversely associated with decongested nasal airway patency, but not with post-β2 FEV1. The adjusted analysis showed that subjects with nasal eosinophilia had a 0.47cm3 decrease in decongested nasal airway patency as compared to subjects without nasal eosinophilia (β-coefficient, -0.47cm3; 95% CI, -0.89 to -0.05; r2=0.14; p=0.03).

Neither FeNO nor total IgE were associated with upper airway or lower airway patency (Table A2, Appendix A).

DISCUSSION

Principal findings

We have shown a significant association between upper and lower airway patency in 6-year-old children from the COPSAC birth cohort. The association was consistent for both baseline values of nasal airway patency and FEV1 and for decongested nasal airway patency and post-β2 FEV1. The association remained significant after adjustments for body size and FVC, and was independent of sex and atopic diseases.

Decongested nasal airway patency was inversely associated with blood eosinophil count and nasal eosinophilia, suggesting its association with upper airway inflammation.

These findings suggest an association between pathophysiology of upper and lower airways.

Other studies

Studies investigating the association between upper and lower airway patency are scarce. One study of 15 adults with perennial allergic rhinitis with moderate-to-severe nasal obstruction and concomitant asthma showed a significant correlation between baseline nasal airflow assessed by anterior rhinomanometry and FEV1(134). Another anterior rhinomanometry study of 300 healthy children aged 8 to11 years reported a significant inverse association between bronchial responsiveness to methacholine and nasal airflow measured both at baseline and after decongestion(135). However, the authors found no association between nasal airflow and FEV1, but did not measure post-β2 FEV1, which reflects irreversible lower airway obstruction(135).

Acoustic rhinometry has been used to show increased nasal mucosal swelling in adults with asthma compared to healthy controls(136). The asthma group had significantly lower FEV1 % predicted, but, unfortunately, there is no available analysis of the association between outcomes of acoustic rhinometry and spirometry in that dataset(136). Besides our data, the association between upper and lower airway patency in preschool children is unexplored.

Meaning of the study

The observed association between upper and lower airway patency could be a reflection of body dimensions, i.e. tall children having larger airways(137) and correspondingly large nasal volumes(107). However, this would be an unlikely explanation of the observed association since we studied children in the narrow age-range of 5-6 years. Furthermore, we adjusted the observed association for FVC and for measurements of body size including height, weight, head circumference and BMI. The association between upper and lower airway patency persisted after such adjustments, assuring the finding is not a simple reflection of body size.

The association between upper and lower airway patency was independent of allergic sensitization, rhinitis and asthma and thereby a consistent finding in both healthy and atopic children. This may reflect a continuous nasobronchial airway inflammation process from healthy to diseased airways, which was supported by the independent associations between blood eosinophil counts, nasal eosinophilia, and upper airway patency. In agreement with this, the thickness of the nasal reticular basement membrane correlates with that of the bronchial tissue in both healthy controls and subjects with coexisting allergic rhinitis and asthma(138). Both respiratory and systemic pathways have been proposed to account for the interaction between upper and lower

airways(70). Loss of the protective functions of the nose may account for the naso-bronchial cross-talk. Alternatively, absorption of inflammatory mediators (e.g. IL-5 and eotaxin) from sites of inflammation into the systemic circulation has been shown to induce release of eosinophils from the bone marrow, prolonged blood eosinophilia, and thereby theoretically systemic propagation of inflammation from nose to lung and visa versa(74). Our finding of a significant association between decongested nasal airway patency (irreversible nasal airway obstruction) and blood eosinophil count is supportive of this hypothesis and suggests that nasal inflammation is not a local phenomenon, but that the entire respiratory tract is involved, even in the absence of clinical asthma.

Nevertheless, blood eosinophil count and nasal eosinophilia were not associated with lower airway patency and neither FeNO nor total IgE were significantly related to upper or lower airway patency. This seems to contradict that the association between upper and lower airway patency reflects common pathology. Alternatively, our data may be interpreted in support of an association between upper and lower airway patency as the physiologic background for the common comorbidity. A possible explanation could be that diminished airway patency contributed to an increased disease propensity.

Strengths and limitations

The major strength of this study is the diagnostic specificity in the COPSAC cohort due to comprehensive prospective investigations based on standard operating procedures and investigator diagnosed clinical end-points following predefined algorithms(92;93) assuring that the observed association between upper and lower airway patency is not due to misclassification. Furthermore, the study is strengthened by the highly standardized objective assessments. Acoustic rhinometry is a non-invasive method for objective measurement of upper airway patency(101), it has been validated against Computed Tomography(34), and the technique is well tolerated by children(36-38). The children of the COPSAC birth cohort are investigated repeatedly from birth(91) and are thus highly trained and cooperative for assessments by acoustic rhinometry and spirometry and all objective measurements are made by trained research assistants at the COPSAC clinical research unit.

The external validity of the study is limited from the setting of a high-risk cohort (all mothers have a history of asthma) as upper and lower airway patency results might differ from the background population. However, an unselected study of 1,735 6-year-old children(137) reported FEV1 values in accordance with our results, and a cross-sectional survey of 137 healthy 5-year-old children(38) presented baseline nasal volumes similar to our findings. Furthermore, our analyses are based on comparisons between upper and lower airway patency within individuals which are unlikely to be affected by increased risk of atopic disease.

A possible technical limitation of the study is the comparison of a static non-physiological measure of upper airway patency (acoustic rhinometry) with a dynamic physiological measure of lower airway patency (spirometry). Theoretically, nasal airflow measured as inspiratory or expiratory peak flow might have been a more reasonable physiological comparator to spirometry. However, the technique requires cooperation beyond what can be expected from a preschool-aged child(31) and nasal peak flow is dependent on both nasal airway patency and the ventilatory capacity of the lungs(139). Furthermore, acoustic rhinometry measures have been shown to correlate well with nasal peak flow(140) and nasal volumes correspond significantly with results obtained by imaging techniques(34;35).

CONCLUSIONS AND PERSPECTIVES

This study shows an association between upper and lower airway patency in children at age 6 years and association between blood eosinophils, nasal eosinophilia, and nasal airway patency. This may support the notion of a common pathophysiology in asthma and allergic rhinitis.

Future research

The significant association between upper and lower airway patency highlights the close link between nose and lung in children already at age 6 years and emphasises that every child presenting either rhinitis or asthmatic symptoms should be suspected of inflammation in the entire respiratory tract. Future studies should address whether young children perceiving nasal symptoms alone show asymptomatic lung function impairment and/or bronchial hyperresponsiveness and whether children with asthma without concurrent rhinitis have objective signs of nasal inflammation.

Importantly, this observational study can only suggest that diminished airway dimensions may predispose to an increased propensity of coexisting asthma and rhinitis. The causal direction between small airways and the development of atopic disease cannot be determined by these data. It is well-known that non-specific bronchial hyperresponsiveness is an important determinant of subsequent development of asthma later in life(131-133). However, it is unknown whether asymptomatic bronchial hyperresponsiveness and/or impaired lung function increase the risk of developing concurrent rhinitis and if asymptomatic irreversible nasal airway obstruction predisposes to development of rhinitis and asthma later in life. The prospective clinical follow-up on the COPSAC birth cohort with visits to the research unit at age 10 and 13 years enables these analyses.

PAPER III

INTRODUCTION

This paper builds on paper I and II as we aimed to further describe differences and similarities between young children with allergic- and non-allergic rhinitis in terms of atopic comorbidity with a special emphasis on asthma and intermediary asthma end-points. We hypothesized that children with allergic- and non-allergic rhinitis exhibit different endotypes of asthma symptoms.

First, children with allergic rhinitis, non-allergic rhinitis, and healthy controls without symptoms of rhinitis were compared for prevalence of asthma, eczema, food sensitization, filaggrin null-mutations, total IgE, blood eosinophil count, FeNO, lung function, reversibility to inhaled β2-agonist, and bronchial responsiveness to cold dry air.

Second, we compared these characteristics in children with allergic- and non-allergic rhinitis, but without concurrent asthma.

Dissimilar associations with intermediary asthma end-points among children with allergic- and non-allergic rhinitis would propose different endotypes of asthma symptoms.

METHODS

Objective measurements

Baseline lung function was assessed by measurement of specific airway resistance (sRaw) by whole body plethysmography(97;98).

Reversibility of airway resistance was determined as the relative change of sRaw 15 minutes after inhaled β2-agonist (two puffs of terbutaline 0.25mg/dose in a pressurized metered dose inhaler with a spacer).

Bronchial responsiveness was determined as the relative change of sRaw 4 minutes after hyperventilating -18˚C cold dry air(102;103). The air was generated by a Respiratory Heat Exchange System (RHES; Erich Jaeger GmbH, Würzburg, Germany). The test was done as a single-step isocapnic hyperventilation test lasting 4 minutes. An animated computer program guided the child to maintain an adequate frequency of breathing aiming at 1 l/min/kg body weight(103) (software available at www.copsac.com). A face mask fitted with a mouthpiece was used during hyperventilation, which ensured mouth breathing and prevented inhalation of room air.

FeNO level was measured by an online technique(99;100) in accordance with recognized guidelines(95). The child was comfortably seated and breathed quietly for about 5 minutes to acclimatize. Thereafter, the child inhaled to near total lung capacity and immediately exhaled at a constant flow of 50 ml/s until a FeNO plateau of ≥2 seconds could be identified. An exhalation lasted at least 4 seconds and the expiratory pressure was maintained at 5–20 cm H2O to close the velum. During exhalation, the child was guided by an exhalation flow driven animated computer program. Two repeated exhalations that agreed within 5% were completed with ≤ 30 seconds intervals and mean FeNO was recorded.

Blood samples were analyzed for eosinophil count, total IgE and specific IgE levels(96). Allergic sensitization was defined as specific IgE ≥0.35kU/L(96;104): allergic sensitization to aeroallergens as any sensitization for cat, dog, horse, birch, timothy grass, mugwort, house dust mites, or moulds; food sensitization as any sensitization for hen's egg, cow's milk, fish, wheat, peanut, soybean, or shrimp.

Nasal eosinophilia was assessed as described under paper I.

Clinical diagnoses

Rhinitis: Allergic- and non-allergic rhinitis were diagnosed by the COPSAC doctors as described in the Methodology section. These definitions were used in the primary analysis.

In a secondary analysis we analyzed (1) allergic rhinitis (rhinitis plus any sensitization to aeroallegens irrespective of association with symptoms) versus non-allergic rhinitis (rhinitis without any sensitization to aeroallergens); (2) allergic rhinitis versus non-allergic rhinitis stratified by presence of nasal eosinophilia; and (3) inflammatory rhinitis (rhinitis plus nasal eosinophilia) versus non-inflammatory rhinitis (rhinitis without nasal eosinophilia).

Current asthma in the 7th year of life was diagnosed as described under paper II.

Eczema ever in the first seven years of life was described by the COPSAC doctors according to predefined morphology and localization at both scheduled and acute visits defined by the Hanifin-Rajka criteria as previously detailed(141;142).

Genetics

Filaggrin genotyping for two independent common null-mutations (R501X and 2282del4) was performed as previously detailed(143). Children were assigned as having a filaggrin mutation if they carried at least one of the mutations.

Statistics

The study group was categorized in three groups: allergic rhinitis, non-allergic rhinitis, and a control group (reference group) without persistent rhinitis symptoms. Odds ratios of asthma, eczema, food sensitization, and filaggrin mutations were calculated by logistic regression, whereas associations between rhinitis diagnoses and continuous outcomes (total IgE, blood eosinophil count, FeNO, baseline sRaw, β2-reversibility, and bronchial responsiveness to cold dry air) were analyzed by generalized linear models expressing results as β-coefficients. Total IgE, blood eosinophil count, and FeNO were log-transformed prior to analysis.

Results are reported with 95% confidence intervals in brackets; a p value ≤0.05 is considered significant. All analyses were done with SAS (v. 9.2).

MAIN RESULTS

Baseline characteristics

Complete follow-up by doctor interview on rhinitis symptoms in the 7th year of life and measurement of specific IgE was available for 290 of the cohort of 411 infants. The study group had increased prevalence of recurrent wheeze in the first 18 months of life (p<0.0001) and higher income (p<0.0001) compared to the group without follow-up on these end-points, whereas there was no differences in eczema, allergic sensitization to aeroallergens, sex, older siblings or family history of allergic rhinitis (Table A3, Appendix A).

Rhinitis was diagnosed in 105 children (36%) and allergic sensitization to inhaled allergens in 76 children (26%). Allergic rhinitis was diagnosed in 38 children (13%) and non-allergic rhinitis in 67 children (23%). Five children classified as non-allergic rhinitis had sensitization to aeroallergens, but no symptoms during exposure. The control group without persistent rhinitis symptoms comprised 185 children (64%).

The overall study group consisted of 142 males (49%). Prevalence of asthma, food sensitization, eczema, nasal eosinophilia, and filaggrin mutations; levels of total IgE, FeNO, and blood eosinophil count; baseline sRaw, reversibility to inhaled β2-agonist, and bronchial responsiveness to cold dry air hyperventilation is described in Table 2.

Association between asthma, eczema and allergic- and non-allergic rhinitis

The relationships between asthma, eczema and allergic- and non-allergic rhinitis are illustrated by the Venn diagrams in Figure 8. The overlapping areas illustrate that current asthma is equally frequent in children with allergic rhinitis (21%) and non-allergic rhinitis (20%). Accordingly, both allergic rhinitis (odds ratio 5.0; 95% CI, 1.8 to 14.0; p=0.002) and non-allergic rhinitis (odds ratio 4.6; 95% CI, 1.9 to 11.4; p=0.001) were significantly associated with current asthma (Table 3). Likewise, asthma was significantly associated with rhinitis symptoms (odds ratio 4.8; 95% CI, 2.1 to 10.8; p<0.001) without evidence of interaction with sensitization to inhaled allergens (p value for interaction 0.87).

The Venn diagrams also show that a history of eczema is a more frequent finding in children with allergic rhinitis as compared to non-allergic rhinitis (66% vs. 43%). The odds ratio of eczema was 2.5 (95% CI, 1.2 to 5.1; p=0.01) for children with

TABLE 2:

	Allergic Rhinitis (N=38)		Non-allergic Rhinitis (N=67)		Controls (N=185)
	+/- Asthma (N=38)	- Asthma (N=30)	+/- Asthma (N=67)	- Asthma (N=54)	+/- Asthma (N=185)
Current asthma % (N)	21% (8)	-	20% (13)	-	5% (9)
Ezcema ever % (N)	66% (25)	63% (19)	43% (29)	41% (22)	44% (81)
Food sensitization* % (N)	47% (18)	43% (13)	13% (9)	17% (9)	17% (31)
Filaggrin mutations† % (N)	24% (9)	28% (8)	10% (7)	7% (4)	9% (16)
Nasal eosinophilia % (N)	28% (9)	27% (7)	8% (5)	6% (3)	2% (4)
Total IgE kU/L median (Q1-Q3)	155 (72–384)	125 (60–133)	30 (11–71)	30 (11–71)	41(17–99)
b-eos 10^9/L median (Q1-Q3)	0.46 (0.3–0.6)	0.43 (0.3–0.6)	0.36 (0.3–0.5)	0.38 (0.3–0.5)	0.30 (0.2–0.5)
FeNO ppb median (Q1-Q3)	15.9 (6.1–29.6)	13.1 (5.8–25.0)	6.8 (5.3–8.8)	7.0 (5.5–9.1)	6.6 (5.1–9.1)
sRaw kPa/s mean (SD)	1.33 (0.26)	1.27 (0.24)	1.33 (0.30)	1.28 (0.25)	1.34 (0.32)
β2-reversibility‡ mean (SD)	0.20 (0.14)	0.16 (0.13)	0.17 (0.13)	0.17 (0.11)	0.17 (0.14)
Cold air challenge§ mean (SD)	0.23 (0.45)	0.22 (0.40)	0.14 (0.22)	0.12 (0.21)	0.09 (0.22)

Phenotypic characteristics of the study group
Definition of abbreviations: IgE=Immunoglobilin E; Q=quartile; b-eos=blood eosinophil count; FeNO=Fractional exhaled Nitric Oxide; sRaw=specific airway Resistance.
**Specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp).*
† Filaggrin null-mutations in R501X or 2282del4
‡The relative change in sRaw before and after bronchodilator
§The relative change in sRaw before and after cold dry air hyperventilation

TABLE 3:

Controls vs:	Allergic Rhinitis		Allergic Rhinitis without asthma		Non-Allergic Rhinitis		Non-Allergic Rhinitis without asthma	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
Current asthma	5.0 (1.8–14.0)	0.002	-	-	4.6 (1.9–11.4)	0.001	-	-
Ezcema ever	2.5 (1.2–5.1)	0.01	2.2 (1.0–4.8)	0.06	1.0 (0.6–1.7)	0.94	0.9 (0.5–1.6)	0.64
Food sensitization*	4.5 (2.1–9.4)	<0.001	3.7 (1.6–8.5)	0.002	0.8 (0.3–1.7)	0.52	1.0 (0.4–2.2)	0.95
Filaggrin mutations†	3.3 (1.3–8.3)	0.01	3.8 (1.4–9.9)	0.01	1.2 (0.5–3.1)	0.69	0.8 (0.3–2.5)	0.69
	β-coefficient (95% CI)	P	β-coefficient (95% CI)	P	β-coefficient (95% CI)	P	β-coefficient (95% CI)	P
Total IgE	1.34 (0.9–1.8)	<0.001	1.13 (0.7–1.6)	<0.001	-0.28 (-0.6–0.1)	0.12	-0.33 (-0.7–0.1)	0.10
b-eos	0.38 (0.1–0.6)	0.01	0.41 (0.1–0.7)	0.01	0.11 (-0.1–0.3)	0.30	0.18 (-0.1–0.4)	0.14
FeNO	0.72 (0.5–1.0)	<0.001	0.62 (0.4–0.8)	<0.001	-0.03 (-0.2–0.1)	0.76	0.01 (-0.2–0.2)	0.93
sRaw	-0.01 (-0.1–0.1)	0.87	-0.05 (-0.2–0.15)	0.32	-0.01 (-0.1–0.1)	0.79	-0.04 (-0.1–0.1)	0.42
β2-reversibility‡	0.03 (-0.02–0.1)	0.24	-0.004 (-0.1–0.1)	0.90	0.0004 (-0.04–0.04)	0.99	0.01 (-0.04–0.1)	0.78
Cold dry air challenge§	0.14 (0.04–0.24)	0.008	0.14 (0.03–0.2)	0.01	0.05 (-0.04–0.1)	0.28	0.04 (-0.1–0.1)	0.38

Comparison of allergic rhinitis with and without asthma, non-allergic rhinitis with and without asthma, and controls.
Definition of abbreviations: OR=Odds Ratio; IgE=Immunoglobilin E; b-eos=blood eosinophil count; FeNO=Fractional exhaled Nitric Oxide; sRaw=specific airway Resistance.
**Specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp).*
† Filaggrin null-mutations in R501X or 2282del4
‡The relative change in sRaw before and after bronchodilator
§The relative change in sRaw before and after cold dry air hyperventilation

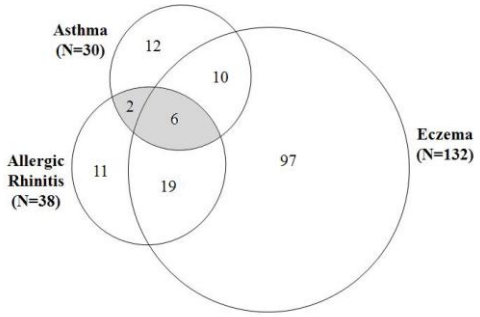
allergic rhinitis and 1.0 (95% CI, 0.6 to 1.7; p=0.94) for children with non-allergic rhinitis (Table 3).

Allergic- vs. non-allergic rhinitis

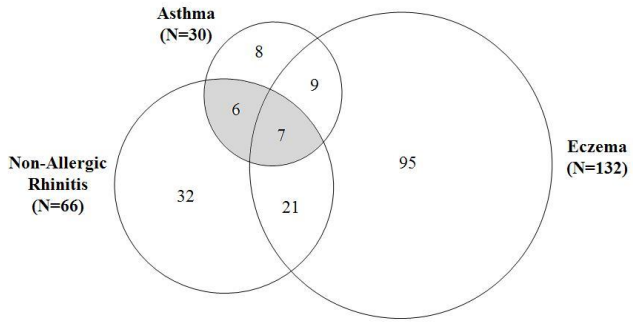
Children with allergic rhinitis compared to non-allergic rhinitis more often had sneezing (79% vs. 58%, p=0.03), nasal rub-bing/itching (66% vs. 40%, p=0.01), itchy/watery eyes (66% vs. 42%, p=0.02), and trials of nasal steroid treatments (37% vs.19%, p=0.05), whereas blocked nose was more prevalent in children with non-allergic rhinitis (76% vs. 58%, p=0.02). Length of the rhinitis history was increased in allergic rhinitis cases as compared

FIGURE 8:

A)



B)



Venn diagrams illustrating the association between asthma, eczema and allergic rhinitis (A), and non-allergic rhinitis (B).
The size of the circles and overlapping areas are area-proportional with respect to the total study population. The overlapping areas between asthma and allergic rhinitis and asthma and non-allergic rhinitis are shaded.

FIGURE 9:

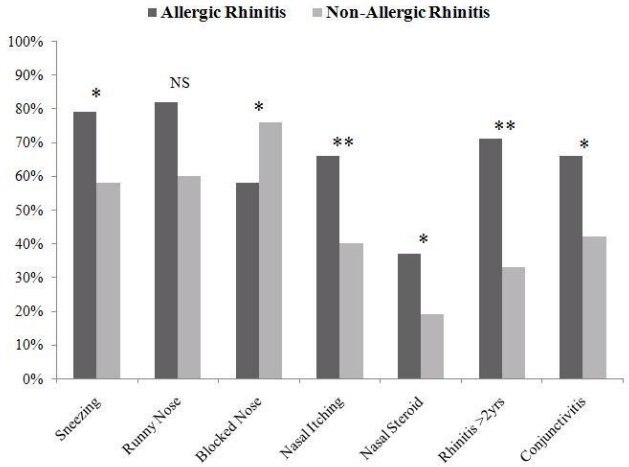


*Diagram showing individual symptom patterns, nasal steroid trials and length of rhinitis history in children with allergic rhinitis compared to non-allergic rhinitis. Comparisons are done with Chi-square statistics; *p≤0.05; **p≤0.01; NS=Non-Significant.*

to non-allergic rhinitis (>2 years duration, 71% vs. 33%, p=0.0002) (Figure 9).

Sensitization to food allergens was present in 47% (N=18) of children with allergic rhinitis, but only in 13% (N=9) of children with non-allergic rhinitis. Allergic sensitization to at least one of the tested food allergens was significantly associated with allergic rhinitis (odds ratio, 4.5; 95% CI, 2.1 to 9.4; p<0.001), but not with non-allergic rhinitis (odds ratio, 0.8; 95% CI, 0.3 to 1.7, p=0.52).

Children with allergic rhinitis had increased levels of total IgE (median values, 155kU/l vs. 41kU/l; p<0.0001), increased blood eosinophil count (median values, 0.46x109/l vs. 0.30x109/l; p=0.01) and elevated FeNO level (median values, 15.9ppb vs. 6.6ppb; p<0.0001) as compared to children without persistent rhinitis. Non-allergic rhinitis subjects were comparable to asymptomatic controls except for the increased asthma prevalence (Table 3).

Children with allergic rhinitis had increased bronchial responsiveness to cold dry air challenge (relative change in sRaw, 23% vs. 9%; p=0.008), whilst non-allergic rhinitis children were comparable to the controls. There were no differences in baseline sRaw or reversibility to inhaled β2-agonist (Table 3).

Allergic rhinitis defined as rhinitis plus any sensitization to aeroallergens (irrespective of relation to symptoms) and non-allergic rhinitis as rhinitis without sensitization did not modify the association with asthma or any of the other findings (Table A4, Appendix A). We found similar associations with asthma and intermediary asthma end-points in allergic rhinitis children with and without nasal eosinophilia as well as in non-allergic rhinitis with and without nasal eosinophilia (Table A5, Appendix A). The analysis of inflammatory vs. non-inflammatory rhinitis was comparable to allergic rhinitis vs. non-allergic rhinitis except that response to cold dry air challenge was not increased in inflammatory rhinitis which is probably due to low numbers (Table A6, Appendix A).

Allergic- vs. non-allergic rhinitis in children with asthma

Asthma in children with allergic rhinitis is compared with asthma in children with non-allergic rhinitis in Table A7, Appendix A. Both children with allergic rhinitis and asthma as well as children with non-allergic rhinitis and asthma had increased baseline sRaw, whereas only children with allergic rhinitis and asthma had elevated FeNO, bronchial hyperresponsiveness, and reversibility to β2-agonist.

Allergic- vs. non-allergic rhinitis in children without asthma

We subsequently studied children with allergic- and non-allergic rhinitis without concurrent asthma. Increased prevalence of eczema and food sensitization, and elevated total IgE and blood eosinophil count were also present in children with allergic rhinitis without concurrent asthma, but not in non-allergic rhinitis. In particular, bronchial responsiveness to cold dry air challenge (relative change in sRaw, 22% vs. 8%; p=0.01) and FeNO level (median values, 13.1ppb vs. 6.6ppb; p<0.001) were increased in children with allergic rhinitis without asthma, but not in children with non-allergic rhinitis (Table 3).

Filaggrin null-mutations

Filaggrin mutations were strongly associated with allergic rhinitis by age 7 (odds ratio, 3.3; 95% CI, 1.3 to 8.3; p=0.01), but not with non-allergic rhinitis (odds ratio, 1.2; 95% CI, 0.5 to 3.1; p=0.69). In order to investigate whether filaggrin mutations explained the

differences between allergic- and non-allergic rhinitis we adjusted all significant associations for filaggrin mutations which did not substantially alter the associations (Table A8, Appendix A).

DISCUSSION

Principal findings

First, asthma coexisted equally frequent in 7-year-old children with allergic- and non-allergic rhinitis from the COPSAC birth cohort of mothers with asthma suggesting a link between asthma and rhinitis beyond an allergy driven mechanism.

Second, children with allergic rhinitis and asthma showed increased FeNO and bronchial hyperresponsiveness, but with no such association in children with non-allergic rhinitis and asthma. This suggests different endotypes of asthma in children with allergic- and non-allergic rhinitis.

Third, children with allergic rhinitis without asthma still exhibited increased bronchial responsiveness and FeNO suggesting that the allergy driven symptoms propagate a disease process in both upper and lower airways even when symptoms only reveal as allergic rhinitis.

Together, these observations support the concept of a close connection between upper and lower airway diseases partly from a common allergy driven process, but equally from non-allergic (unknown) mechanisms.

Meaning of the study

Increased prevalence of asthma was present in both children with allergic- and non-allergic rhinitis suggesting a link between symptoms from upper and lower airways beyond allergy driven mechanisms. In paper II, we demonstrated that upper and lower airway patency are strongly associated in children with allergic- and non-allergic rhinitis(144). Thus, generalized diminished airway dimensions may contribute to an increased propensity of coexisting rhinitis and asthma possibly explained by shared genetic variants. In support of a non-allergic communality between upper and lower airway diseases, a large proportion of adults with chronic rhinosinusitis have lung function abnormalities and report having asthma(145;146). Nasal symptoms are also frequently reported by patients with chronic obstructive pulmonary disease and bronchiectasis(145;147;148).

Our finding of a similar asthma prevalence in children with allergic- and non-allergic rhinitis is in accordance with two previous studies in 5-year-old(11) and 10-year-old children(21), but at variance with studies of adolescents and adults consistently showing higher prevalence of asthma in subjects with allergic rhinitis as compared to non-allergic rhinitis(20;22;23). These findings suggest that a proportion of children with allergic rhinitis will develop asthma later in life which is consistent with longitudinal data from a recent report confirming allergic rhinitis as a determinant of adult-onset asthma(65). Additionally, some children with non-allergic rhinitis may turn allergic later in life. Both hypotheses support the ARIA recommendations(2) of testing for asthma in all children presenting symptoms of persistent rhinitis either allergic or non-allergic.

We also found that children with allergic rhinitis and asthma had increased prevalence of bronchial hyperresponsiveness and elevated FeNO in comparison to children with non-allergic rhinitis and asthma which suggests different endotypes of asthma symptoms in children with allergic- and non-allergic rhinitis. Previous studies have shown bronchial hyperresponsiveness in one third to one half of adults with allergic rhinitis(55;149), but there are only few reports comparing the allergic- and non-allergic rhinitis phenotypes. One comparative study of mixed adults and adolescents reported airway hyperresponsiveness in a significantly greater proportion of subjects with allergic rhinitis as compared to non-allergic rhinitis(22), which was confirmed in a recent survey of 10-year-olds with allergic- and non-allergic rhinitis diagnosed from questionnaires and assessments of allergic sensitization(21). Our findings demonstrate different endotypes of asthma symptoms in young children with allergic- and non-allergic rhinitis and warrant increased awareness of children with coexisting asthma and allergic rhinitis as this phenotype is characterized by raised values of FeNO and bronchial hyperresponsiveness already at age 7. Further studies in preschool-aged children with allergic- and non-allergic rhinitis comparing prevalence of intermediary asthma end-points are needed(6).

Children with allergic rhinitis, but without asthma still exhibited increased bronchial responsiveness and elevated FeNO level, which have also been demonstrated in adults(79;82) and school-aged children(21). In preschool-aged, two cross-sectional studies of non-asthmatic children with allergic rhinitis have also shown increased prevalence of bronchial hyperresponsiveness(83;84). However, interpretation of the former is limited by a broad age-range (6-15 years)(84) and the latter by a subjective "auscultative" evaluation of response to methacholine challenge(83). Our findings suggest a sub-clinical bronchial disease process in young children with allergic rhinitis and emphasize allergic rhinitis as a marker of a generalized airway disease(65;150). This interpretation is supported by studies showing that nasal allergen challenge can cause bronchial inflammation(76;151) and that segmental bronchial provocation induces a nasal inflammatory response(77). These findings may play an important role for the follow-up of children with allergic rhinitis without clinical asthma as asymptomatic bronchial hyperresponsiveness is described in association with subsequent development of asthma later in life(131-133). Assessment of nasal eosinophilia does not seem to help the clinician to identify rhinitis children at particular risk of asthma as nasal eosinophilia was not a frequent finding in this age group nor was nasal eosinophilia associated with any of the intermediary asthma end-points.

As expected(20-22), the allergic rhinitis phenotype had increased prevalence of eczema, food sensitization, increased total IgE, and elevated blood eosinophil count, whereas these characteristics were not associated with the non-allergic rhinitis phenotype. In addition, filaggrin loss-of-function mutations were strongly associated with allergic rhinitis, but not with non-allergic rhinitis. We have previously shown that filaggrin mutations are associated with the development of eczema(141) and allergic sensitization(88), and others have reported an association with allergic rhinitis(152). Therefore, a higher frequency of filaggrin mutations in children with allergic rhinitis could have accounted for the differences between the allergic- and non-allergic rhinitis phenotypes. However, adjusting the analysis for filaggrin mutations did not modify our findings assuring that the associations with asthma and intermediary asthma end-points are not driven by differences in filaggrin genotypes.

Strengths and limitations

A major strength of the study is the high diagnostic accuracy and sensitivity in this closely monitored birth cohort with comprehensive objective assessments and daily diary cards(91-93). All diagnoses were made by the doctors employed at the COPSAC re-

search unit, not the family practitioner, minimizing risk of misclassification. Rhinitis diagnosis was based on parent interviews (not questionnaires) allowing validation and interpretation of the symptom history. Similarly, asthma was diagnosed based on clinical assessments according to a predefined algorithm and daily diaries, not questionnaires. The diaries were reviewed by the COPSAC doctors at six-monthly clinical sessions and immediately upon onset of any respiratory symptom reducing risk of recall bias. Furthermore, the study is strengthened by the comprehensive objective assessments of atopic biomarkers, lung function, bronchial reversibility and responsiveness, performed in accordance with standard operating procedures by highly trained research assistants at the COPSAC research unit.

The clinical follow-up rate of the cohort by age 7 of 70% with clinical information on rhinitis, asthma, eczema, sensitization to food allergens, levels of total immunoglobulin E (IgE), blood eosinophil count, nasal eosinophilia, fractional exhaled nitric oxide (FeNO), measures of lung function, and bronchial responsiveness is also a significant strength of the study.

The principal limitation of the study is the setting of a birth cohort of mothers with a history of asthma which diminishes the generalizability of our findings. However, population based studies of adolescents and adults with rhinitis have shown associations in agreement with our findings(20;22).

We found that non-allergic rhinitis was twice as common as allergic rhinitis which is different from studies of adults where the proportion of subjects with non-allergic rhinitis is one third to one fourth of the rhinitis population(20;22;23). It may be speculated that a sub-clinical allergic diathesis exists in a proportion of children with non-allergic rhinitis. “Entopy” – presence of nasal mucosal, but not systemically specific IgE – may explain early steps in the development of allergic rhinitis(129;153). However, this remains speculative and the evidence from our data shows that children with rhinitis without established sensitization often have concurrent asthma.

Alternatively, misclassification may have occurred. However, we found well described differences in symptom presentation between children with allergic- and non-allergic rhinitis(2) and reanalyzing data as allergic rhinitis (rhinitis plus any sensitization to aeroallergens irrespective of relation to symptoms) versus non-allergic rhinitis (rhinitis without any sensitization) as well as inflammatory rhinitis (rhinitis plus nasal eosinophilia) versus non-inflammatory rhinitis did not modify our findings.

CONCLUSIONS AND PERSPECTIVES

Asthma was equally frequent in children with allergic- and non-allergic rhinitis suggesting a link between upper and lower airway diseases beyond an allergy driven mechanism. Children with allergic rhinitis, but not non-allergic rhinitis, had increased bronchial responsiveness and FeNO suggesting different endotypes of asthma associated with allergic- and non-allergic rhinitis. Children with allergic rhinitis without asthma also exhibited bronchial hyperresponsiveness and raised FeNO suggesting a sub-clinical bronchial disease process and supporting the allergic disease process to involve both upper and lower airways. These observations lend support to a close connection between upper and lower airway diseases partly from an allergy driven process, but equally from non-allergic mechanisms.

Future research

A future study should be performed in a non-selected population to investigate whether our findings are specific for offspring of atopic mothers. The possible role of mono- versus polysensitization, absolute specific IgE levels, seasonal versus perennial versus mixed triggers, and ARIA severity classes of rhinitis should also be addressed to determine if these phenotypic characteristics associate with specific endotypes of asthma in young children with rhinitis.

It is plausible that shared genetic variants contribute to the communality between symptoms of rhinitis and asthma. Large-scale (meta) genome wide associations studies (GWAS) of children with coexisting asthma and rhinitis may uncover novel SNPs, genetic networks, single susceptibility genes and subsequently new biological pathways involved in the development of concurrent asthma and rhinitis. Such insight could be utilized and further explored in a translational systems biology approach assessing nasal gene expression (mRNA patterns) in nasal scrapes obtained with Rhinoprobes®, and proteomics (nasal mediator levels) by the Synthetic Absorptive Matrix method. Identification of novel disease endotypes and biomarkers may enable distinguishing groups with more uniform responses to treatment and the results might be translated into clinical practice including disease prevention, diagnosis and individualized treatments with improved efficacy, reduced risk of side-effects and more cost-effective medicines.

GWAS identification of polymorphisms that are associated with increased risk of symptoms from both nose and lung may help defining how genetic constitution and expression of individual genes or networks associate with development of concurrent asthma and rhinitis in childhood. By combining information from such analyses with the few well recognized risk variants of disease (e.g filaggrin(143), ORMDL3(87), and DENND1B(154) variants), it may be possible to predict accurately each individual infant’s genetic risk for disease. In particular, genotyping of high-risk infants early in life may improve primary prevention of disease as well as identification of children at increased risk of developing atopic comorbidities such as asthma.

Finally, it would be interesting to employ another data analysis strategy such as explorative cluster analysis gathering genomic, proteomic, clinical and environmental data. By focusing on quantitative and qualitative information directly related to the status of the child, solid and unforeseen associations are likely to emerge possibly leading to classification of novel endotypes of disease.

SUMMARY

Allergic- and non-allergic rhinitis are very common diseases in childhood in industrialized countries. Although these conditions are widely trivialized by both parents and physicians they induce a major impact on quality of life for the affected children and a substantial drainage of health care resources.

Unfortunately, diagnostic specificity is hampered by nonspecific symptom history and lack of reliable diagnostic tests which may explain why the pathology behind such diagnoses is poorly understood. Improved understanding of the pathophysiology of allergic- and non-allergic rhinitis in young children may contribute to the discovery of new mechanisms involved in pathogenesis and help direct future research to develop correctly timed preventive measures as well as adequate monitoring and treatment of children with rhinitis.

Asthma is a common comorbidity in subjects with allergic rhinitis and epidemiological surveys have suggested a close connection between upper and lower airway diseases expressed as the "united airways concept". Furthermore, an association between upper and lower airway diseases also seems to exist in non-atopic individuals. Nevertheless, the nature of this association is poorly understood and there is a paucity of data objectivizing this association in young children.

The aim of this thesis was to describe pathology in the upper and lower airways in young children from the COPSAC birth cohort with investigator-diagnosed allergic- and non-allergic rhinitis.

Nasal congestion is a key symptom in both allergic- and non-allergic rhinitis, and eosinophilic inflammation is a hallmark of the allergic diseases. In paper I, we studied nasal eosinophilia and nasal airway patency assessed by acoustic rhinometry in children with allergic rhinitis, non-allergic rhinitis and healthy controls. Allergic rhinitis was significantly associated with nasal eosinophilia and irreversible nasal airway obstruction suggesting chronic inflammation and structural remodeling of the nasal mucosa in children already at age 6 years. Non-allergic rhinitis exhibited no change in the nasal airway patency, but some nasal eosinophilia albeit less than children with allergic rhinitis. These findings suggest different pathology in allergic- and non-allergic rhinitis which may have important clinical implications for early pharmacological treatment of rhinitis in young children.

In paper II, we utilized the nasal airway patency end-points derived from paper I to examine whether upper and lower airway patency are associated. Upper airway patency was assessed by acoustic rhinometry before and after intranasal α-agonist and lower airway patency by spirometry before and after inhaled β2-agonist. Upper and lower airway patencies were strongly associated and independent of body size, rhinitis and asthma. The association was consistent for both baseline values and for decongested nasal airway patency and post-β2 FEV1. Blood and nasal eosinophilia were also associated with nasal airway obstruction. This suggests generalized diminished airway dimensions as a novel susceptibility factor for concurrent symptoms of asthma and rhinitis in early childhood and supports the notion of a common pathophysiology in asthma and rhinitis. The clinical interpretation of these findings is that all children presenting either rhinitis or asthma should be considered inflamed in the entire respiratory tract.

In paper III, we aimed to describe asthma and intermediary asthma end-points associated with allergic- and non-allergic rhinitis in preschool-aged children. At age 7 years, we evaluated prevalence of asthma, eczema, food sensitization, and filaggrin mutations; levels of total IgE, FeNO, and blood-eosinophils; lung function and bronchial responsiveness to cold dry air. We found that asthma was similarly associated with allergic- and non-allergic rhinitis suggesting a link between upper and lower airway diseases beyond an allergy associated inflammation. Only children with allergic rhinitis had increased bronchial responsiveness and elevated FeNO suggesting different endotypes of asthma symptoms in young children with allergic- and non-allergic rhinitis. We also found bronchial hyperresponsiveness and raised values of FeNO in children with allergic rhinitis without asthma suggesting sub-clinical bronchial inflammation and supporting the allergic disease process to involve both upper and lower airways.

In conclusion, these observations objectively show marked differences in nasal pathology in young children with allergic- and non-allergic rhinitis and lend support to a close connection between upper and lower airway diseases partly from an allergy driven process, but equally from non-allergic mechanisms.

LIST OF ABBREVIATIONS

ARIA	Allergic Rhinitis and its Impact on Asthma
BMI	Body Mass Index
COPSAC	COpenhagen Prospective Studies on Asthma in Childhood
DOHaD	Developmental Origins of Health and Disease
FEF25-75	Forced Expiratory Flow at 25–75% of the pulmonary volume
FeNO	Fractional exhaled Nitric Oxide
FEV1	Forced Expiratory Volume in the first second
FVC	Forced Vital Capacity
GINA	Global INitiative for Asthma
GWAS	Genome Wide Associations Studies
IgE	Immunoglobulin E
IL	InterLeukin
ISAAC	International Study of Asthma and Allergy in Childhood
MCA1	Minimum Cross-sectional Area at 0-2.2 cm from nares
MCA2	Minimum Cross-sectional Area at 2.2-5.4 cm from nares
NARES	Non-Allergic Rhinitis with Eosinophilia Syndrome
NO	Nitric Oxide
SAM	Synthetic Absorptive Matrix
sRaw	specific airway resistance
Th2	T-helper 2
VOL1-4	Nasal VOLume from 1 to 4 cm from nares

APPENDIX A – ADDITIONAL RESULTS

TABLE A1:

Estimated change in nasal reversibility to α-agonist* (%) per 1% increase in bronchial reversibility to β2-agonist†			
Crude association		**Adjusted association‡**	
β-coefficient (95% CI)	**P**	**β-coefficient (95% CI)**	**P**
0.15 (-0.19 to 0.49)	0.39	0.10 (-0.25 to 0.45)	0.57

Association between reversibility of the upper and lower airways

**Nasal reversibility = ((Decongested nasal airway patency-Baseline nasal airway patency)/ Baseline nasal airway patency) x100.*

†Bronchial reversibility = ((Post-β2 FEV1-FEV1)/FEV1) x100.

‡Adjusted for sex, height, weight, head circumference, BMI, FVC, allergic sensitization, rhinitis, asthma, nasal steroid usage and inhaled steroid usage.

TABLE A2:

	Decongested nasal airway patency, cm3		Post β2-FEV1, L	
	β-coefficient (95% CI)	**P**	**β-coefficient (95% CI)**	**P**
Eosinophil count, 10^9/L	-0.42 (-0.77 to -0.07)	0.02	0.05 (-0.03 to 0.13)	0.21
Nasal eosinophilia	-0.47 (-0.89 to -0.05)	0.03	0.03 (-0.05 to 0.11)	0.47
FeNO, ppb	0.003 (-0.03 to 0.03)	0.82	-0.0001 (-0.01 to 0.01)	0.98
Total IgE, 100kU/L	0.02 (-0.03 to 0.06)	0.43	0.003 (-0.01 to 0.01)	0.53

Associations between upper and lower airway patency and blood eosinophil count, FeNO and total IgE.
The models are corrected for sex, nasal steroid usage and inhaled steroid usage.

TABLE A3:

Characteristic	Studygroup N=290	Dropouts N=121	P
Male sex	142 (49%)	60 (50%)	0.79*
Recurrent wheeze, 0-1½yrs	22 (8%)	0	<0.0001†
Eczema, 0-1½yrs	99 (34%)	33 (27%)	0.17*
Allergic sensitization to aeroallergens, 1½yrs	10 (4%)	4 (5%)	0.75†
Siblings at birth	118 (41%)	34 (35%)	0.26*
Parental income at birth			<0.0001*
<400.000dKr	74 (26%)	38 (38%)	
400.000-600.000dKr	138 (48%)	45 (46%)	
>600.000dKr	74 (26%)	16 (16%)	
Father allergic rhinitis	94 (34%)	32 (28%)	0.29*
Mother allergic rhinitis	222 (77%)	88 (73%)	0.49*

Dropout analysis.
**Chi-square test; †Fisher's exact test*

TABLE A4:

Controls vs: N=185	Allergic Rhinitis N=43		Allergic Rhinitis without asthma N=34		Non-Allergic Rhinitis N=62		Non-Allergic Rhinitis without asthma N=50	
Binary Variables	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**
Current asthma	5.0 (1.8–13.4)	0.002	-	-	4.6 (1.8–11.5)	0.001	-	-
Ezcema ever	2.4 (1.2–4.8)	0.01	2.0 (1.0–4.3)	0.07	0.9 (0.5–1.7)	0.80	0.8 (0.4–1.6)	0.59
Food sensitization*	4.3 (2.1–8.8)	<0.0001	3.8 (1.7–8.4)	0.001	0.6 (0.3–1.5)	0.31	0.8 (0.3–1.9)	0.61
Filaggrin mutations†	2.8 (1.2–6.9)	0.02	-	-	1.3 (0.5–3.4)	0.56	-	-
Continous Variables	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**
Total IgE	1.24 (0.8–1.7)	<0.0001	1.08 (0.6–1.5)	<0.0001	-0.34 (-0.7–0.02)	0.07	-0.41(-0.8– -0.02)	0.04
B-eosinophils	0.39 (0.1–0.6)	0.002	0.42 (0.1–0.7)	0.004	0.09 (-0.1–0.3)	0.44	0.15 (-0.1–0.4)	0.23
FeNO	0.59 (0.4–0.8)	<0.0001	0.71 (0.5–0.9)	<0.0001	-0.09 (-0.2–0.1)	0.52	-0.04 (-0.2–0.2)	0.71
sRaw	-0.02 (-0.1–0.1)	0.68	-0.06 (-0.2–0.04)	0.23	-0.003 (-0.1–0.1)	0.94	-0.03 (-0.1–0.1)	0.54
β2-reversibility‡	0.03 (-0.02–0.08)	0.20	0.001 (-0.1–0.1)	0.97	-0.004 (-0.05–0.04)	0.87	0.004 (-0.04–0.1)	0.87
Cold dry air challenge§	0.12 (0.02–0.2)	0.02	0.11 (0.02–0.2)	0.02	0.05 (-0.03–0.1)	0.22	0.04 (-0.04–0.1)	0.33

Comparisons of allergic rhinitis and non-allergic rhinitis with and without asthma and controls.
Allergic rhinitis is defined as rhinitis plus sensitization to any tested aeroallergen; non-allergic rhinitis as rhinitis without sensitization to aeroallergens.
**Specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp).*
† Filaggrin null-mutations in R501X or 2282del4
‡The relative change in sRaw before and after bronchodilator
§The relative change in sRaw before and after cold dry air hyperventilation

TABLE A5:

Controls vs: N=165	Allergic Rhinitis with nasal eosinophilia N=9		Allergic Rhinitis without nasal eosinophilia N=23		Non-Allergic Rhinitis with nasal eosinophilia N=5		Non-Allergic Rhinitis without nasal eosinophilia N=54	
Binary Variables	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**
Current asthma	4.9 (0.9–27.0)	0.07	3.6 (1.0–12.8)	0.05	5.7 (0.5–60.4)	0.15	3.9 (1.5–10.2)	0.01
Ezcema ever	2.5 (0.6–10.2)	0.21	3.5 (1.3–9.3)	0.01	4.9 (0.5–45.0)	0.16	0.8 (0.5–1.6)	0.60
Food sensitization*	4.1 (1.0–16.2)	0.05	3.9 (1.6–9.9)	0.004	3.4 (0.5–21.4)	0.19	0.6 (0.2–1.6)	0.35
Filaggrin mutations†	3.3 (0.6–17.5)	0.16	5.4 (1.9–15.6)	0.002	2.9 (0.3–27.7)	0.36	1.2 (0.4–3.5)	0.77
Continous Variables	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**
Total IgE	0.82 (-0.02–1.7)	0.06	1.18 (0.6–1.7)	<0.0001	-0.62 (-1.7–0.5)	0.27	-0.30 (-0.7–0.1)	0.13
B-eosinophils	0.26 (-0.2–0.8)	0.31	0.38 (0.04–0.7)	0.03	-0.10 (-0.8–0.6)	0.78	0.17 (-0.1–0.4)	0.18
FeNO	0.93 (0.5–1.3)	<0.0001	0.57 (0.3–0.9)	0.0002	0.27 (-0.3–0.8)	0.33	-0.06 (-0.2–0.1)	0.54
sRaw	-0.14 (-0.4–0.1)	0.18	0.04 (-0.1–0.2)	0.54	-0.09 (-0.4–0.3)	0.63	-0.02 (-0.1–0.1)	0.71
β2-reversibility‡	0.02 (-0.1–0.1)	0.69	0.03 (-0.04–0.1)	0.38	-0.03 (-0.2–0.1)	0.68	0.01 (-0.03–0.06)	0.73
Cold dry air challenge§	0.21 (0.1–0.4)	0.01	0.16 (0.04–0.3)	0.01	0.15 (-0.1–0.4)	0.26	0.03 (-0.1–0.1)	0.48

Comparisons of allergic rhinitis and non-allergic rhinitis with and without nasal eosinophilia and controls.
**Specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp)*
† Filaggrin null-mutations in R501X or 2282del4
‡The relative change in sRaw before and after bronchodilator; §The relative change in sRaw before and after cold dry air hyperventilation

TABLE A6:

Controls vs: N=165	Inflammatory Rhinitis N=14		Non-Inflammatory Rhinitis N=77	
Binary Variables	**Odds Ratio (95% CI)**	**P**	**Odds Ratio (95% CI)**	**P**
Current asthma	5.1 (1.2–22.0)	0.03	3.8 (1.6–9.2)	0.003
Ezcema ever	3.1 (0.9–10.2)	0.07	1.3 (0.7–2.2)	0.40
Food sensitization*	3.8 (1.2–11.9)	0.02	1.3 (0.7–2.7)	0.40
Continous Variables	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**
Total IgE	0.30 (-0.4–1.0)	0.41	0.14 (-0.2–0.5)	0.43
B-eosinophils	0.14 (-0.3–0.6)	0.51	0.23 (-0.02–0.4)	0.13
FeNO	0.71 (0.4–1.0)	<0.0001	0.10 (-0.1–0.3)	0.27
sRaw	-0.13 (-0.3–0.1)	0.16	0.0004 (-0.1–0.1)	0.99
β2-reversibility†	0.01 (-0.1–0.1)	0.90	0.01 (-0.03–0.1)	0.47
Cold dry air challenge‡	0.05 (-0.10–0.19)	0.54	0.07 (-0.01–0.15)	0.07

Comparison of inflammatory rhinitis, non-inflammatory rhinitis and controls.
Inflammatory rhinitis is defined as rhinitis with nasal eosinophilia; non-inflammatory rhinitis as rhinitis without nasal eosinophilia.
**Specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp).*
†The relative change in sRaw before and after bronchodilator
‡The relative change in sRaw before and after cold dry air hyperventilation

TABLE A7:

Controls vs: N=169	Asthma and Allergic Rhinitis N=8		Asthma and Non-Allergic Rhinitis N=13	
Variables	**β-coefficient (95% CI)**	**P**	**β-coefficient (95% CI)**	**P**
FeNO	0.40 (0.03–0.78)	0.03	-0.12 (-0.46–0.21)	0.47
Baseline sRaw	0.25 (0.05–0.45)	0.01	0.17 (0.01–0.33)	0.04
β2-reversibility*	0.18 (0.08–0.28)	0.001	0.01 (-0.07–0.09)	0.80
Cold dry air challenge†	0.18 (0.02–0.37)	0.05	0.12 (-0.04–0.29)	0.13

Comparison of asthma in children with allergic rhinitis and non-allergic rhinitis versus controls.
**The relative change in sRaw before and after bronchodilator; †The relative change in sRaw before and after cold dry air hyperventilation*

TABLE A8:

	Allergic Rhinitis vs. Controls				Non-allergic Rhinitis vs. Controls			
	Crude		Adjusted for FLG* mutations		Crude		Adjusted for FLG* mutations	
Binary Variables	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**	**OR (95% CI)**	**p**
Current asthma	5.0 (1.8-14.0)	0.002	5.7 (2.0-16.8)	0.001	4.6 (1.9-11.4)	0.001	5.1 (2.0-13.0)	0.001
Ezcema ever	2.5 (1.2-5.1)	0.01	2.0 (1.0-4.3)	0.07	-	-	-	-
Food sensitization†	4.5 (2.1-9.4)	<0.001	4.4 (2.0-9.6)	<0.001	-	-	-	-
Continous Variables	**β-coefficient (95% CI)**	**p**	**β-coefficient (95% CI)**	**p**	**β-coefficient (95% CI)**	**p**	**β-coefficient (95% CI)**	**p**
Total-IgE	1.34 (0.9-1.8)	<0.001	1.33 (0.9-1.8)	<0.001	-	-	-	-
B-eosinophils	0.38 (0.1-0.6)	0.01	0.31 (0.04-0.6)	0.02	-	-	-	-
FeNO‡	0.62 (0.4-0.8)	<0.001	0.60 (0.4-0.8)	<0.001	-	-	-	-
Cold dry air challenge§	0.14 (0.04-0.24)	0.008	0.15 (0.04-0.25)	0.007	-	-	-	-

Significant characteristics of allergic and non-allergic rhinitis adjusted for filaggrin null-mutations.
**FLG= Filaggrin null-mutations in R501X or 2282del4.*
†Food sensitization=specific IgE ≥0.35kU/l for at least one of 7 food allergens (hen's egg, cow's milk, fish, wheat, peanut, soybean, shrimp).
‡FeNO=Fraction of exhaled Nitric Oxide; §Cold dry air challenge=the relative change in sRaw before and after cold dry air hyperventilation.

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