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Insights into Wheeze and Asthma across the Life Course

by

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ABSTRACT

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INSIGHTS INTO WHEEZE AND ASTHMA ACROSS THE LIFE COURSE

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Wheeze and asthma are major health problems worldwide, affecting all age groups. Severe asthma and asthma exacerbations represent particular problems because they are associated with high morbidity and healthcare costs.

This thesis used data collected as part of the EuroPrevall and UBIOPRED studies to provide new insights into wheeze and asthma across the life course. Areas explored included risk factors for preschool wheeze, the relationship between atopy and disease severity and risk factors for exacerbations in patients with severe asthma/preschool wheeze.

The EuroPrevall birth cohort consisted of 12,049 infants from nine European countries. Data on wheeze in the second year of life was available in 8775 (72.8%). The prevalence of wheeze varied considerably across Europe, ranging from 1.7% in Lodz (Poland) to 17.2% in Reykjavik (Iceland). Risk factors for wheeze in the second year of life included lower respiratory tract infections, postnatal maternal smoking, day care attendance and male gender. However, their importance varied between centres suggesting that unique risk factors operate in different countries.

In the UBIOPRED study, participants with mild to moderate and severe asthma/preschool wheeze were recruited into adult, school and preschool age cohorts. At baseline, a detailed asthma and allergic disease history was taken. Skin prick testing, specific IgE measurement and component resolved allergen diagnostics (ISAC Chip®) were performed. The severe cohorts were followed up after 12-18 months. Clinical clusters and allergic sensitisation clusters were generated. The prevalences of allergic disease and allergic sensitisation did not differ significantly according to asthma/wheeze severity in any age group. A history of previous exacerbations and poor asthma control were risk factors for future exacerbations across the life course. Rates of prospective exacerbations did not differ between clinical or allergic sensitisation clusters. Further research is needed to determine whether novel biomarkers can more accurately predict asthma outcomes than clinical parameters.

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DECLARATION OF AUTHORSHIP

I, **Anna Selby** declare that this thesis and the work presented in it are my own and has been generated by me as the result of my own original research.

Insights into Wheeze and Asthma across the Life Course

I confirm that:

1. This work was done wholly or mainly while in candidature for a research degree at this University;

2. Where any part of this thesis has previously been submitted for a degree or any other qualification at this University or any other institution, this has been clearly stated;

3. Where I have consulted the published work of others, this is always clearly attributed;

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6. Where the thesis is based on work done by myself jointly with others, I have made clear exactly what was done by others and what I have contributed myself;

Data collection for the EuroPrevall and UBIOPRED studies was completed prior to my involvement in these studies. My roles included data management, analysis and interpretation. All analyses included in this thesis were undertaken by myself with guidance from my supervisor, statisticians and other team members.

7. Parts of this work have been published as:

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Definitions and Abbreviations

95% CI= 95% confidence intervals

ACQ= Asthma Control Questionnaire

ACT= Asthma Control Test

AD= Atopic dermatitis

ALSPAC= Avon Longitudinal Study of Parents and Children

ANOVA= Analysis of variance

API= Asthma Predictive Index

aOR= Adjusted odds ratio

AQLQ= Asthma Quality of Life Questionnaire

aRR= Adjusted relative risk

ATS= American Thoracic Society

BAMSE= Barn Allergi Miljö Stockholm Epidemiologi (The Stockholm Children Allergy and Environmental Study)

BIB= Born in Bradford

BMI= Body mass index

BP= Blood Pressure

BTS= British Thoracic Society

BUD= Budesonide

C-ACT= Children's Asthma Control Test

CAMP=Childhood Asthma Management Program

CARE= Childhood Asthma Research and Education

CI= Confidence interval

COAST= Childhood Origins of Asthma

CRD= Component resolved diagnostics

CT= Computed tomography

DBPCFC= Double-blind, placebo-controlled food challenge

EAACI= European Academy of Allergy and Clinical Immunology

eCRF= Electronic case report form

ECRHS= European Community Respiratory Health Survey

ED= Emergency Department

eNO= Exhaled nitric oxide

ERS= European Respiratory Society

FAMD= Factor analysis for mixed data

FEF_{25-75}= Forced expiratory flow at 25—75% of vital capacity

FEV_1= Forced expiratory volume in 1 second

FOT= Forced oscillation technique

FP= Fluticasone propionate

FVC= Forced vital capacity

GINA= Global Initiative for Asthma

GINIplus= German Infant Nutritional Intervention Study plus influence of pollution and genetics on allergy development

GORD= Gastro-oesophaeal reflux disease

GP= General Practitioner

HADS= Hospital Anxiety and Depression Scale

HC= Healthy controls

hMPV= Human Metapneumovirus

HR= Heart rate

ICS= Inhaled corticosteroids

ICU= Intensive Care Unit

iFAAM= Integrated Approaches to Food Allergen and Allergy Risk Management

IgA= Immunoglobulin A

IgE = Immunogulin E

IL= Interleukin

IMI= Innovative Medicines Initiative

INMA= INfancia y Medio Ambiente (Childhood and Environment Project)

IRR= Incidence rate ratio

ISAAC=International Study of Asthma and Allergies in Childhood

ISAC= Immuno Solid-phase Allergen Chip

ISU= International scientific units

KU=Thousand Units

LISAplus= Influence of lifestyle factors on the development of the immune system and allergies in East and West Germany plus the influence of traffic emissions and genetics

LRTI= Lower respiratory tract infection

LTRA= Leukotriene receptor antagonist

MAAS= Manchester Asthma and Allergy Study

MARS= Medication Adherence Report Scale

MAS= Multicentre Allergy Study

MedALL= Mechanisms of the Development of Allergy

MMA= School aged children with severe asthma

MMAn= Non-smoking adults with mild to moderate asthma

MMW= Preschool children with mild to moderate wheeze

NAC= National Asthma Campaign

NCICAS= National Cooperative Inner City Asthma Study

OCS= Oral corticosteroids

OR= Odds ratio

PACQLQ= Paediatric Asthma Caregiver's Quality of Life Questionnaire

PAM= Partition around medoids

PARIS= Pollution and Asthma Risk in Infant Study

PAQLQ= Paediatric Asthma Quality of Life Questionnaire

PIAMA= Prevention and Incidence of Asthma and Mite Allergy

PC_{20}= Provocative concentration causing a 20% fall in FEV_1

PEF= Peak expiratory flow

PICU= Paediatric Intensive Care Unit

QOL= Quality of life

RHEA= Mother-Child Cohort in Crete

ROBBIC= Rome and Bologna Birth Italian Cohorts

RR= Respiratory rate

RSV=Respiratory syncytial virus

SA= School aged children with severe asthma

SAn= Non-smoking adults with severe asthma

SAs/ex= Smokers/ex-smokers with severe asthma

SARP= Severe Asthma Research Program

SCORAD= Scoring Atopic Dermatitis Assessment

SD= Standard deviation

SE= Standard error

SIGN= Scottish Intercollegiate Guidelines Network

SNOT20= Sino-Nasal Outcomes Test

SOP= Standard operating procedure

SPT= Skin prick test

STRA= Severe therapy resistant asthma

SWS= Southampton Women's Survey

TENOR= The Epidemiology and Natural History of Asthma : Outcomes and Treatment Regimens

Th2= T-helper 2

TNF-α= Tumour necrosis factor-alpha

UBIOPRED= Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes

URTI= Upper respiratory tract infection

URECA= Urban Environment and Childhood Asthma

VmaxFRC= Maximal expiratory flow at functional respiratory volume

Chapter 1: Introduction

Wheezing episodes associated with respiratory tract infections are common in early childhood[1] affecting around 50% of children by the age of six years.[2] Most infants who wheeze do not have persistent symptoms. However, in some infants wheeze predisposes to chronic asthma.[2] Asthma affects an estimated 300 million people worldwide[3] and is the most common lower respiratory disease in children.[4] It is a heterogenous condition characterised by varying degrees of bronchoconstriction, chronic airway inflammation and hyperresponsiveness. Clinical manifestations include episodic wheeze, cough, shortness of breath and chest tightness.[4,5] Sensitisation to common aeroallergens plays a key role in the pathogenesis of most cases of childhood asthma.[6] When these allergens are processed by airway dendritic cells, a T-helper-2 (Th2) cell response results in the production of IL-4, IL-5 and IL-13. This promotes the formation of specific IgE antibodies, which bind to receptors on the surfaces of mast cells and basophils. Cross-linking of IgE on allergen exposure, subsequently leads to the release of inflammatory mediators such as IL-1 and tumour necrosis factor-alpha (TNF-α), which cause bronchoconstriction, airway oedema and increased mucus production.[6] Recent research suggests that in asthma the airway epithelium is structurally and functionally defective, facilitating abnormal responses to inhaled allergens.[7] Other factors which contribute to the development of asthma include early exposure to certain viruses such as rhinovirus and respiratory syncytial virus (RSV), genetics and exposure to tobacco smoke.[4,8]

Both preschool wheeze and asthma place a substantial burden on healthcare resources and impair quality of life.[4,9,10] In 2004, for example, it was estimated that in the UK, 75,000 hospital admissions per year are due to asthma, a quarter of which are in children less than four years of age.[3] Patients with severe asthma and those who experience frequent exacerbations have particularly high levels of morbidity and consume a disproportionate amount of healthcare resources.[11,12] Research priorities in asthma therefore include improving our understanding of the aetiology of preschool wheeze, [4] identifying modifiable risk factors for exacerbations and defining the characteristics of those with severe asthma in more detail.

1.1 Early Childhood Wheeze

1.1.1 Wheeze Phenotypes

Many researchers have attempted to group children with preschool wheeze into different categories and develop tools to predict which children will develop asthma.[9] A landmark study by Martinez et al. (The Tucson Children's Respiratory Study) identified different wheeze phenotypes based on the timing of onset and duration of wheeze: transient early wheeze (wheeze during the first 3 years of life only), late-onset wheeze (wheeze starting after the age of 3 years) and persistent wheeze (wheeze during the first 3 years of life and at 6 years of age).[2] Similar phenotypes have been seen in other longitudinal studies including the Avon Longitudinal Study of Parents and Children (ALSPAC) and the Prevention of Infant Asthma and Mite Allergy (PIAMA) study.[13] Although widely used in epidemiological studies, these phenotypes are not useful in clinical practice because they can only be applied retrospectively. [10] Therefore, classification of children with wheeze according to the temporal pattern of their symptoms has also been proposed. According to the European Respiratory Society (ERS), episodic wheeze is defined as wheeze in discrete episodes associated with viral respiratory tract infections, with no wheeze between episodes. Multi-trigger wheeze is defined as wheeze associated with viral respiratory tract infections, but also in response to other triggers such as smoke and allergen exposure. A limitation of this classification is that there is considerable overlap between groups, they are not stable phenotypes and they do not reliably predict long term prognosis. [14]

Although only around 40% of children who wheeze in the first few years of life continue to wheeze at school age,[2,15] persistent lung function deficits have been demonstrated in those with transient early wheeze.[2,15,16] Martinez et al., for example, demonstrated that children with transient early wheeze had significantly lower $V_{max}FRC$ values at 6 years of age compared to those who had never wheezed and those with late onset wheeze (1097.7 ml/sec versus 1262.1 ml/sec and 1174.9 ml/sec, respectively).[2] Similarly, children in the Southampton Women's Survey (SWS) with transient early wheeze had statistically lower mean FEV_1 % predicted (100.3 versus 103.1, $p<0.05$) and FEF_{25-75} % predicted (95.1% versus 100.3, $p<0.05$) values at six years than children who had never wheezed.[16] In both studies, children with persistent wheeze had lower lung function parameters than all other groups, including children with early transient wheeze.[2,16] Others have also shown that 'definite bronchial hyperresponsiveness' is more prevalent amongst persistent wheezers (42.6%) than non-wheezers (10.7%, $p<0.001$) and early transitent wheezers (15.1%, $p<0.001$). These findings suggest that preschool wheeze is not a benign disorder and that early intervention is important to prevent later morbidity.[15]

Using data from the Tucson Children's Respiratory Survey, Castro-Rodriguez et al. developed two Asthma Predictive Indices (APIs) based on recurrent episodes of wheezing during the first three years of life and five other criteria: two major (parental physician-diagnosed asthma and physician-diagnosed eczema) and three minor (physician-diagnosed allergic rhinitis, wheeze without colds and peripheral eosinophilia ≥4%). A stringent API was defined three or more episodes of wheeze per year and at least one major or two minor criteria, whilst a loose API was defined as less than 3 episodes of wheeze per year and at least one major or two minor criteria. [17] A limitation of these and other predictive tools, which have since been developed, is that they lack sensitivity and have poor positive predictive values.[1,9,18] According to Castro-Rodriguez et al., for example, the sensivity of a positive stringent API score at 3 years of age for asthma between the ages of 6 to 13 years is only 16%.[18] Furthermore, they have only been validated for the populations in which they were derived [9] and there is no evidence that initiating preventative treatment in children at high risk of asthma modifies the natural course of the disease. [19-21] Guilbert et al., for example, randomised 285 children aged 2 to 3 years with a positive API to treatment with fluticasone propionate 88 μg twice daily or masked placebo for two years, followed by a one-year period of observation without medication.[19] During the observation year, there were no significant differences between the groups in the proportion of episode-free days (86.8% versus 85.9%, p=0.78), the number of exacerbations or lung function.[19] Episode free days were defined as days with no asthma-like symptoms, no unscheduled visits for respiratory symptoms and no use of supplementary asthma medications and exacerbations were defined as the need for a course of prednisolone to control asthma-like symptoms. A possible explanation for these results is that in asthma the airways are abnormal at inception and therefore, prevention is not possible once symptoms have developed.[21] If so, identification of modifiable, early life risk factors for wheeze is of paramount importance.

1.1.2 Prevalence Studies

The rise in asthma prevalence during the last three decades of the twentieth century was considered too rapid to be due to genetic factors alone. Therefore, the International Study of Asthma and Allergies in Childhood (ISAAC)[22] and European Community Respiratory Health Survey (ECRHS)[23,24] were established to examine international prevalence patterns of asthma symptoms and identify environemental factors operating at a population level.[22] Identifying environmental risk factors for disease is important because these offer the greatest opportunity for prevention.

ISAAC, which commenced in 1991, comprised three phases:

- Phase one- This used written questionnaires in 156 centres across 56 countries to describe the prevalence of asthma, eczema and allergic rhinitis. Two age groups were studied: children aged 6-7 years and children aged 13-14 years.
- Phase two- This investigated potential aetiological factors contributing to the international differences observed in phase one.
- Phase three- This was a repetition of phase one to allow time trends in prevalence to be assessed.

Phase one included a total of 721,601 children: 463,801 children aged 13-14 years from 155 centres across 56 countries and 257,800 children aged 6-7 years from 91 centres across 38 countries. Large variations in the prevalence of asthma symptoms were seen in both age groups. The prevalence of 'asthma ever' in the 13-14 years age group, for example, ranged from 1.6 to 28.2% and the proportion of participants reporting wheeze in the past 12 months ranged from 2.1 to 32.2%. Similar findings were seen in the younger age group though the prevalence of most symptoms was lower. Prior to ISAAC, the greatest number of countries included in any study comparing geographical variations in the prevalence of childhood asthma was four. [25-27]

The ECRHS, which was established in 1988, included only adults aged 20-44 years. 138,565 participants were recruited from 48 centres across 22 countries, predominantly in Western Europe. As in ISAAC, higher rates of asthma symptoms were seen in English-speaking countries i.e. the UK, Australia, New Zealand and the USA. Other findings common to ISAAC and the ECRHS were a West to East gradient within Europe and inter-country variation in prevalence rates. [28]

To date, only one study has evaluated variations in the prevalence of preschool wheeze across different countries. However, this study used data from ten idependent Mechanisms of the Development of ALLergy (MeDALL) cohorts in eight different countries.[29] Comparing data from individual studies is difficult due to use of different defintions. Furthermore, temporal trends may account for differences between studies conducted more than ten years apart. By comparing the prevalence of preschool wheeze between countries and analysing differences in environmental exposures, it may be possible to identify new risk factors and determine the importance of those which are already known. [30]

1.1.3 Risk Factors for Early Childhood Wheeze

Over the past 30 years, more than 130 birth cohorts focusing on allergy and asthma have been established.[31] Birth cohort studies allow the temporal relationship between exposures and disease onset to be explored, providing valuable insights into disease causality.[31] Numerous risk factors for early childhood wheeze have been identified including male gender, prenatal and postnatal smoke exposure and contact with other children.

1.1.3.1 Respiratory Tract Infections

Respiratory tract infections, particularly those due to respiratory syncytial virus (RSV) and rhinoviruses, have consistently been implicated in the pathogenesis of childhood wheezing.[9,32,33] When Kusel et al. collected respiratory secretions from 263 infants on all occasions of acute respiratory illness in the first year of life, they found that 39% of wheezy lower respiratory infections were attributable to rhinoviruses and 12% to RSV.[34] Human metapneumovirus (hMPV) is also an important pathogen with Wolf et al. demonstrating that among children under the age of 5 who had been hospitalised with hMPV or RSV infection, wheeze was a presenting feature in over 50% of cases.[35] Furthermore, early infection with rhinovirus or RSV has been linked to wheeze later in childhood.[9] Within the Childhood Origins of Asthma (COAST) cohort, for example, wheezing illnesses caused by rhinovirus infection in the first year of life were the strongest predictor of wheeze during the third year of life (OR 6.6, $p < 0.0001$).[36] Regarding RSV, Sigurs et al. compared the outcomes of 52 infants receiving hospital treatment for RSV bronchiolitis with 93 age matched controls. Amongst those with bronchiolitis and family history of asthma, 38% had asthma at 7.5 years compared to none of the controls with a family history of asthma.[37]

Given that day care attendance increases exposure to respiratory infections,[38] it is not surprising that this is also associated with an increase in airway symptoms in early life.[38-40] In the Pollution and Asthma Risk: an Infant Study (PARIS) birth cohort, for example, the strongest predictor of transient wheeze was day care attendance during the first six months of life (OR 3.13, 95% CIs 2.19-4.47, $p < 0.05$) with more than one older sibling also being an important risk factor (OR 1.42, 95% CIs 1.15-1.76, $p < 0.05$).[40] Meanwhile, in the PIAMA birth cohort, children attending daycare in the first two years of life (early daycare) were twice as likely to experience wheezing in the first year of life compared to children not attending daycare (aOR 1.89, 95% CIs 1.50-2.39).[38] The association between early daycare attendance and wheeze did however diminish with increasing age. Other studies have suggested that day care attendance may protect against asthma in later childhood. Ball et al., for example, demonstrated that children with at least two older siblings or who attended day care during the first six months of life were more likely to have frequent wheezing at 2 years of age than children who had less contact with other children (aRR 1.4, 95%

CI 1.1-1.8, p=0.01). However, they were less likely to have frequent wheezing between the ages of 6 (aRR 0.8, 95% CIs 0.8-1.0, p=0.01) and 13 years (aRR 0.3, 95% CI 0.2-0.5, p <0.001). [39] Nicolaou et al. also investigated the relationship between day care attendance and position in sibship with childhood wheeze. They found that entering daycare between 6 and 12 months or after 12 months of age significantly reduced the risk of wheeze at 5 years of age (odds ratio 0.25, 95% CI 0.11-0.60 and 0.65, 0.44-0.98).[41] These results may reflect the fact that early childhood wheeze is predominantly driven by viral respiratory tract infections, whereas asthma at school age is associated with allergic sensitisation.

1.1.3.2 Smoke Exposure

The Tucson Children's Respiratory Study identified maternal smoking in early childhood as an important risk factor for transient early wheeze (aOR 2.2, 95% CIs 1.3-37).[2] Many studies have since confirmed that postnatal passive smoke exposure has deleterious effects on respiratory health in early childhood.[42,43] Differentiating the effects of smoking during and after pregnancy is difficult because few women change their smoking habits after delivery.[44] Studies have, however, demonstrated that maternal smoking during pregnancy is an independent risk factor for wheeze[44,45] and that there may be critical time periods of exposure.[30,46,47] This is biologically plausible given that nicotine restricts fetal growth and disrupts alveolar architecture.[30,44,48] Within the Norwegian Mother and Baby (MoBa) study, maternal smoking during pregnancy was associated with an increased risk of wheeze at age 6-18 months independent of postnatal smoke exposure (aOR 1.13, 95% CIs 1.03-1.29). [47] Maternal smoking during pregnancy conferred a smiliar risk for wheeze between 18 and 30 months (aOR 1.19, 95% CIs 1.02-1.39) in the ALSPAC study.[46] Furthermore, in a pooled analysis of data from eight European birth cohort studies, which included 21,600 children, the risks of both wheeze (aOR 1.39, 95% CI 1.08-1.77) and asthma (aOR 1.65, 95% CI 1.18-2.31) at 4 to 6 years of age were increased in those exposed to cigarette smoke by their mothers during pregnancy.[44] This analysis also found that maternal smoking during the first trimester only (but not the third trimester or during the first year of life) was associated with an increased risk of wheeze (aOR, 95% CI 1.00-2.12) and asthma (aOR 2.10, 95% CI 1.38-3.21).[44] Conversely, the Generation R study (a population based prospective birth cohort study of over 6000 children in Rotterdam) found that only continuous maternal smoking during pregnancy was associated with an increased risk of wheezing between the ages of 1 and 4 years (OR 2.19, 95% CIs 1.24-3.86, p <0.01 for frequent wheezing at age 3).[45]

1.1.3.3 Infant Feeding Practices

The impact of early feeding practices on the development of atopic disease and wheeze has been extensively investigated. Although reviews of the literature have concluded that exclusive breastfeeding for at least four months appears to protective, controversy remains. [49,50] Given the immunologic complexity of breast milk, its influence on the development of disease may differ between individuals. A protective effect of breastfeeding is biologically plausible given that breast milk contains secretory IgA, which provides passive immunity against infections. [49] However, some components of breast milk such as IL-4, IL-5 and IL-13, which are involved in IgE production, may promote the development of allergic disorders and infant wheeze.[50]

A group from Sweden (Kull et al.) prospectively followed up 4089 infants at 1, 2 and 4 years of age. They found that children exclusively breastfed for four months or more had less asthma (defined as at least three reported episodes of wheezing during the first two years of life, in addition to respiratory symptoms treated with inhaled steroids or signs of hyperreactivity without ongoing upper respiratory infection) by 2 years of age compared to those exclusively breastfed for less than 4 months (7.7% versus 12%, OR 0.66, 95% CI 0.51 to 0.87). A similar result was seen when comparing those partially breastfed for six months or more with those partially breastfed for less than six months (7.7% versus 12%, OR=0.69, 95% CI 0.52 to 0.91).[51] At 4 years of age, the risk of asthma was also lower in those exclusively breastfed for four months or more compared to those breastfed for shorter periods (6.4% versus 9.1%, OR=0.72, 95% CI 0.53 to 0.97). In sub-group analysis, the protective effect of breastfeeding tended to be stronger in those with heredity for allergic diseases. However, this interaction was not statistically significant.[52] Oddy et al. collected data on infant feeding in a birth cohort of 2602 Australian children followed up to 6 years of age. They found that the risk of asthma (defined as physician diagnosed asthma and wheeze in the past year) was higher in those exclusively breastfed for less than four months (OR 1.28, 95% CI 1.01 to 1.62, p=0.038). This relationship was not altered by atopy or maternal asthma.[53]

In the 1980s, around 670 infants were recruited into the Dundee Infant Feeding Study. [54,55] They were closely followed up for 24 months after birth and were subsequently reviewed at 7 years of age. The age when infants were introduced to solids (before 8 weeks, between 8-12 weeks or after 12 weeks) had no influence on the incidence of wheeze during the first two years of life.[54] However, solid feeding before 15 weeks was found to increase the probability of wheeze by 7 years of age (21.0% vs 9.7%). In a population based, prospective birth cohort study of 642 children Zutavern et al. also explored whether the age when children are introduced to solids influences the risk of wheeze in early childhood. They found that late introduction of solids (after

3 months) did not protect against preschool wheezing (wheeze at age 5 and in one of the first four years of life) or transient wheezing (wheeze in the first two years of life only). This finding was consistent across different food groups.[56] One study has, however, suggested that delayed introduction of solids may increase the risk of wheeze. Snjiders et al. analysed data from 2258 infants enrolled in a prospective birth cohort study in the Netherlands.[57] They found that the risk of recurrent wheeze (at least four attacks in the first two years of life) was higher in children who first received foods other than cow's milk products between 4-6 months compared to children who received other foods in the first 3 months of life (OR 1.71, 95% CI 1.00-2.95). The risk of recurrent wheeze was even higher in those who first received other foods after 7 months (OR 3.52, 95% CI 1.42-8.73).

A lack of agreement between studies looking at the relationship between infant feeding practices and early childhood wheeze may be due due to use of different definitions for wheeze phenotypes and exclusive breastfeeding. Kull et al., for example, defined exclusive breastfeeding as the period that infants were only breastfed and that no formula, cow's milk or solid foods had been introduced,[51] whereas Oddy et al. defined exclusive breastfeeding as the age in months that other milk was introduced (without taking the age that solids were introduced into account).[53] Furthermore, in many of the aforementioned studies data were collected retrospectively introducing the potential for recall bias. Large multi-centre studies using prospectively collected data are therefore needed to clarify the role of early feeding practices in the development of wheezing disorders.

1.2 Atopy and Allergic Diseases

1.2.1 The Relationship between Atopy and Asthma

Atopy refers to a genetic predisposition to become sensitised and produce IgE antibodies in response to ordinary exposure to allergens.[58] It is conventionally defined as a positive (≥ 0.35 kU/l) serum allergen-specific IgE (sIgE) or a positive skin prick test (wheal diameter ≥ 3mm) to at least one common inhalant or food allergen.[59] IgE sensitisation does not necessarily mean that an individual will have allergic signs and symptoms. However, as demonstrated by observational and epidemiological studies atopy is closely linked with the development and expression of asthma.[60]

Within a whole population birth cohort of 1,456 children on the Isle of Wight, skin prick testing was performed at 4 years of age. Current wheeze (wheeze in the last 12 months) and currently diagnosed asthma (current wheeze and ever-diagnosed asthma) were subsequently recorded at 10 years of age when bronchial hyperresponsiveness was measured. After adjusting for numerous factors including family history, respiratory infections and parental smoking, atopy was identified as a risk factor for current wheeze (OR 3.69, 95% CI 2.36-5.76, p <0.001), currently diagnosed asthma (OR 7.22, 95% CI 4.13-12.62, p <0.001) and bronchial hyperresponsiveness (OR 5.4, 95% CI 3.06-9.47, p <0.001).[61] Atopy was also identified as a risk factor for wheeze within the German Multicenter Allergy Study (MAS).[62] MAS is a prospective cohort study, which recruited 1314 infants at birth in five German cities and followed them up for 20 years at 19 time points.[63] Specific IgE levels to a range of food allergens and inhalant allergens were measured at 1,2,3,5,6 and 7 years of age. From birth to 5 years, the frequency of wheezing episodes was similar in those with atopic and non-atopic wheeze. However, after the age of 5 years the course of wheezing differed markedly between the two groups. 90% of those with wheeze but no atopy became asymptomatic by 13 years of age compared with only 56.2% of those with atopic wheeze (p=0.0002) (Figure 1).[62] Furthermore, perennial allergic sensitisation (detectable IgE (≥ 0.35kU/l) to house dust mite, cat and dog dander) in the first 3 years of life was associated with impaired lung function at school age. The MAS group have suggested that the risk of asthma at school age is influenced by the timing of atopic sensitisation based on the finding that in atopic children with asthma at 7 years of age, atopic sensitisation occurred significantly earlier than in atopic children without asthma (39.4% before age 1 year vs 21.0%, p=0.015).[64]

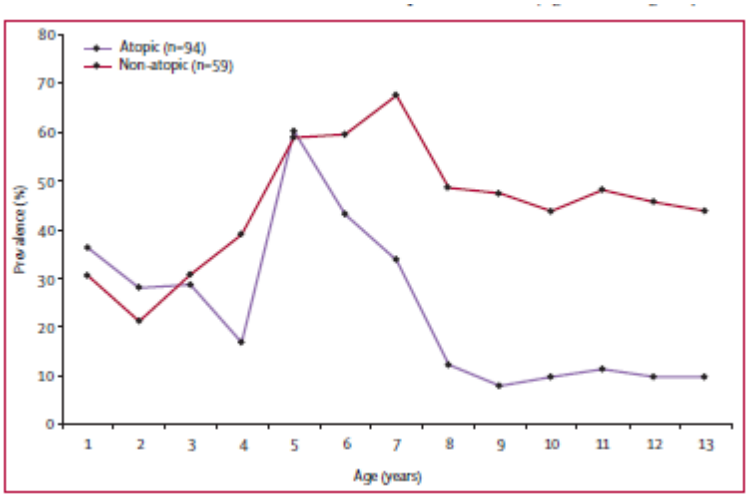

Figure 1 Prevalence of Wheeze from Birth to 13 Years in Children with Any Wheezing Episode at School Age (5-7 years), according to Atopic Status [62 (p.766)]

Data supporting an association between atopy and asthma are more limited in adults. Recently, however, Warm et al. examined the prevalence and impact of sensitisation to airborne allergens on asthma and allergic rhinitis among 737 adults (age 21-86 years).[65] They found that sensitisation to any allergen was associated with current asthma (OR 2.94, 95% CI 1.81-4.77, p<0.001) and current rhinitis (OR 5.31, 95% CI 3.53-7.99). For allergic rhinitis, this association remained when subjects were analysed separately in three age groups (22-40 years, 41-60 years and 61-86 years) but was considerably stronger in subjects aged 22 to 40 years (OR 19.84, 95% CI 7.24-54.40) compared to those older than 40 years. For asthma, an association with sensitisation to any allergen was only seen in those aged 22-40 years (OR 3.53, 95% CI 1.27-9.81) and those aged 41-60 years (OR 3.17, 95% CI 1.59-6.31). Another noteworthy finding of this study is that the prevalence of allergic sensitisation among subjects with asthma varied according to age of asthma onset. Of those who developed asthma before the age of 6 years, 86% were sensitised in adulthood, whereas only 26% of subjects who developed asthma in adulthood (≥20 years) were sensitised.[65] The prevalence of atopy and risk factors for asthma were also investigated in over 5000 adults (mean age 29.6 years, range 18-45 years) whose children were enrolled in the National Asthma Campaign Manchester Allergy and Asthma Study ([NAC]MAAS). 9.7% of participants had a current physician diagnosis of asthma. Sensitisation to dust mite, cat, dog and mixed grasses were all independently associated with asthma, whilst the risk of current asthma was considerably higher in those with a greater number of positive skin prick tests to these four allergens (OR 4.3, 95% CI 3.3-5.5 for any two allergens compared to 10.4, 95% CI 7.7-14 for any four allergens).[66]

In summary, atopy is associated with asthma in both children and adults. However, the association appears to be stongest in school age children and in those who are sensitised to multiple allergens.

1.2.1.1 Quantification of Atopy

Other analyses undertaken by the MAAS study group have demonstrated that quantification of atopy (either by the level of specific IgE, the size of skin test wheals or the number of positive tests) may better predict wheezing and reduced lung function than information on the presence or absence of atopy.[59,67] Among 521 children enrolled in MAAS, the risk of wheeze at 5 years of age increased with increasing specific IgE to to dust mite, cat and dog ($p < 0.0001$ for all).[68] Meanwhile, in a random sample of 983 parents (31.7% with asthma), increasing levels of specific IgE to these three allergens were significantly associated with lower FEV_1 levels ($p < 0.001$ for all). Similar findings were seen using the size of wheal on skin prick testing as a continuous variable, with significantly poorer lung function with increasing wheal size.[67] An association between asthma severity and the degree of atopy has also been proposed. Carroll et al. enrolled 400 children (age 7-18 years) with asthma in a multicentre asthma genetics study.[69] An algorithm was used to score asthma severity, skin prick testing to a panel of 7 aeroallergens was performed and total IgE levels were measured. Although the summative SPT wheal size was not associated with asthma severity score, it was associated with hospitalisation in the previous year ($p < 0.001$), inhaled corticosteroid use ($p < 0.001$) and evidence of airways obstruction ($p < 0.001$).[69] Furthermore, Sharples et al. have reported that a large mean wheal diameter to aeroallergens on SPT is a feature of severe therapy-resistant asthma (STRA). In 31 children with difficult asthma the mean wheal diameter was 10.5mm compared to 17mm in 46 children with STRA ($p=0.026$).[70]

1.2.1.2 Sensitisation Patterns

It has also been proposed that atopy encompasses multiple sub-phenotypes which relate to asthma in different ways. Lazic et al., for example, used a machine learning approach to cluster children in the MAAS and Isle of Wight birth cohorts into different classes of atopic sensitisation based on skin prick testing and specific IgE results.[71] Five classes, which were very similar between the two cohorts, were described. These included a class of non-sensitised children and a class of children with sensitisation to a wide range of allergens. Children in the latter class were much more likely to have asthma in both the MAAS (aOR 20.1, 95% CI 10.9-40.2) and the Isle of Wight (aOR 11.9, 95% CI 7.3-19.4) cohorts. Conventional atopy also predicted asthma but much lower odds ratios were reported (aOR 5.5, 95% CI 3.4-8.8 in MAAS and 5.8, 95% CI 4.1-8.3 in the Isle of Wight cohort).

Component resolved diagnostics (CRD) allows detection of specific IgE to individual proteins within whole allergen sources. It is hoped that the emergence of this will further improve our understanding of the relationsip between allergic sensitisation and disease.[72] The ImmunoCAP ISAC chip (ThermoFisher Scientific, Uppsala, Sweden) is a biochip which enables measurement of IgE antibodies to 112 components from 51 allergen sources in a single step.[73] Prosperi et al. performed allergen screening using this in 461 children aged 11 years participating in a population-based birth cohort study.[74] Using a variety of methods including logistic regression and non-linear statistical learning models, it was possible to discriminate asthma and rhino-conjunctivitis with reasonable accuracy on the basis of allergic sensitisation patterns (area under the receiver operating characteristic curve = 0.76-0.82). The same study group subsequently used latent variable modelling to identify patterns of component-specific IgE responses and relate these to asthma, eczema and hay fever.[72] 61 allergen components were clustered into 3 component groups (CG1, CG2 and CG3) each including different protein families. Sensitisation to CG3 (which comprised 27 components of plant, animal and fungal origin) was most strongly associated with asthma (OR 8.20, 95% CI 3.49-19.24, $p<0.001$) whilst sensitisation to CG1 (which comprised 27 components of plant origin) was most strongly associated with hay fever (OR 12.79, 95% CI 6.84-23.9, $p<0.001$). For eczema, there was no significant association between sensitisation to any of the componenet groups.

To date, these are the only studies which have explored the clinical significance of different patterns of component sensitisation. Further studies, which include patients of different ages and consider asthma severity are therefore needed. The relationship between different patterns of component sensitisation and the risk of asthma exacerbations could also be explored.

1.2.1.3 The Atopic March

Atopic dermatitis (eczema), allergic rhinitis and food allergy are also linked to atopy. Birth cohort studies have reported age-related differences in the prevalence these of conditions (Figure 2). This has led to the concept of the atopic march, which is defined as the natural progression from atopic dermatitis in early childhood to asthma and allergic rhinitis in later childhood.[75]

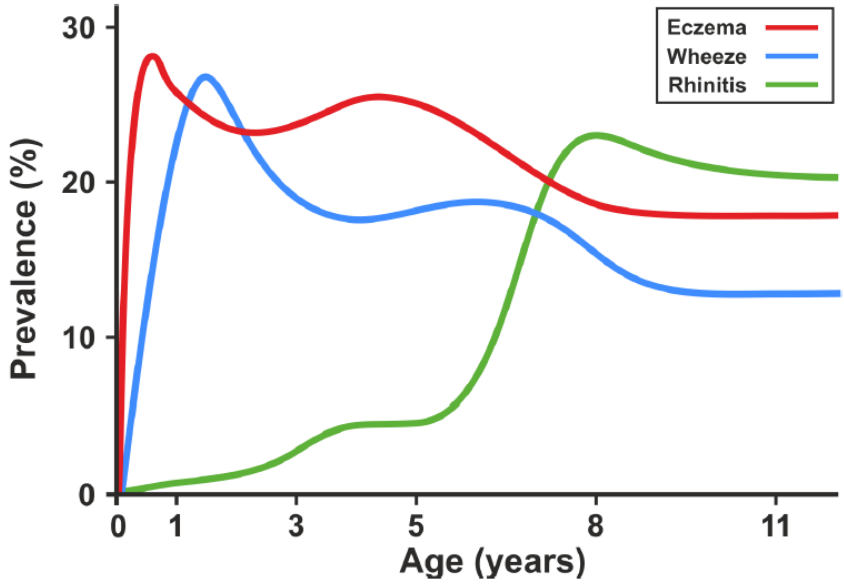

Figure 2 Cross-sectional Change in the Prevalence of Wheeze, Eczema and Rhinitis in the MAAS and ALSPAC Cohorts [75 (p.12)]

In support of the atopic march, the Tucson Children's Respiratory Study found that children with eczema in the first two years of life were more likely to have persistent wheeze at age 6 (OR 2.4, 95% CI 1.3-4.6).[2] Similarly, within a whole population birth cohort on the Isle of Wight, eczema at age 4 was a risk factor for asthma at age 10 (OR 2.15, 95% CI 1.24-3.73).[76] There is also evidence linking early eczema and allergic rhinitis. Within the MAS cohort, for example, early atopic dermatitis (before 2 years of age) was found to be a risk factor for allergic rhinitis at 7 years of age (aOR 2.5, 95% CI 1.4-4.6, p=0.0024).[77] However, other findings from the Multicenter Allergy Study challenge the concept of the atopic march. Illi et al., for example, demonstrated that although early atopic dermatitis is associated with wheeze at age 7 (OR 1.93, 95% CI 1.22-3.06), this is not the case after adjusting for early wheeze and early atopic sensitisation (aOR 1.46, 95% CI 0.73-2.90). It was therefore proposed that children with atopic dermatitis and wheeze are more likely to represent a distinct phenotype rather than a progression of atopic diseases.[78] van der Hulst et al. performed a systematic review to assess the risk of developing asthma in children with atopic eczema during the first 4 years of life. Across four birth cohort studies, the pooled risk of asthma at 6 years of age or older was 2.14 (95% CI was 1.67-2.75) in children with atopic eczema.

However, it was highlighted that only 1 in 3 children with atopic eczema acutally develop asthma, suggesting a more complex relationship between atopic eczema and asthma than described by the atopic march.[79]

The existence of the atopic march has also been questioned on the basis that in most studies the development of eczema, allergic rhinitis and asthma has been examined at population level using cross-sectional data.[75] To address this, Belgrave et al. used a machine learning approach to model the development of allergic conditions in the ALSPAC and MAAS cohorts taking into account the chronology of symptoms in individual patients. Eight different temporal classes describing the trajectories of eczema, wheeze and rhinitis during childhood were identified. 51.2% of participants had no symptoms. Six of the symptomatic classes were characterised by the presence of only one or two conditions, with less than 7% of those with symptoms and 3.1% of all participants following a trajectory resembling the atopic march.[75] Even when participants with mild eczema were excluded, the atopic march was only seen in 5.8% of participants. Once again, this suggests that associations between atopic disorders may be due to shared gene-environment interactions rather than a progressive march. The association of food allergy with other allergic disorders and its role in the atopic march remains uncertain and requires further investigation.[80]

1.2.2 Food Allergy

Food allergy is defined as an adverse reaction to food mediated by an immunologic mechanism, involving specific IgE (IgE-mediated), cell-mediated mechanisms (non-IgE-mediated) or both (mixed IgE- and non-IgE-mediated).[81] The true prevalence of food allergy is difficult to determine because few studies utilise double-blind, placebo-controlled food challenges (the gold standard of diagnosis). A systematic review published in 2014 reported that the lifetime prevalence of self-reported food allergy in Europe is 17.3% compared to a point prevalence of challenge diagnosed food allergy of only 0.9%.[82] The most common allergenic foods are cow's milk, hen's egg, soy, wheat, peanuts, tree nuts, fish and shellfish. Milk, egg, soy and wheat allergies tend to present in infancy with most children becoming tolerant by school age. However, other allergies typically present later in childhood and usually persist into adulthood.[83] [84]

1.2.2.1 Diagnosing Food Allergy

A detailed clinical history is essential in the diagnosis of food allergy.[81] Skin prick testing and measurement of serum specific IgE may help to support a diagnosis of food allergy. However, IgE sensitisation can occur without clinical symptoms. Skin prick testing is usually deemed positive if a wheal diameter of 3mm or greater is produced in the presence of a negative saline control and a positive histamine control .[83,84] This gives a sensitivity of 70-100% but a specificity of only 40-

70%.[81] Using a cut-off of 0.35 kU/l, specific IgE also has a high sensitivity, making it a useful test to rule out IgE-mediated food allergy.[84] However, the false positive rate tends to be even higher than that of skin prick testing.[85] The specificity of skin prick testing and specific IgE can be improved by using higher cut-off values. Sporik et al., for example, correlated the results of skin prick testing and open challenges to milk, egg and peanut in 467 children referred to a tertiary allergy clinic for the evaluation of suspected food allergy. They found that above a wheal diameter of 8mm for cow's milk, 7mm for egg and 8mm for peanut, negative reactions did not occur i.e. the specificity of skin prick testing was 100%.[86] Given that cutaneous reactivity appears to be influenced by age, gender, time of day and season, these values may, however, differ between populations.[87] Similarly, Sampson et al. compared the reults of specific IgE (generated using the Pharmacia CAP method) with the outcomes of oral food challenges in 196 children and adolescents. They found that above levels of 6KU/l for egg, 32 kU/l for milk, 15 kU/l for peanut and 20kU/l for fish, specific IgE could predict clinical reactivity with ≥95% certainty.[88] Other studies have, however, reported different specific IgE cut-off levels above which 95% of children show clinical reactivity. Van Venn et al., for example, reported that amongst 280 children recruited from primary and secondary care settings, a positive predicitive value of 95% for peanut allergy was not even achieved with a specific IgE level greater than 100 kU/l and that the relationship between peanut allergy and peanut-specific IgE is stongly influenced by eczema.[89] These findings demonstrate that skin prick testing and specific IgE cannot reliably differentiate between asymptomatic sensitisation and clinical allergy, especially if there is not a clear history of symptoms following ingestion of a single food.[83]

A food challenge may be undertaken where there is diagnostic uncertainity or to demonstrate oral tolerance to a food. Food challenges involve giving increasing amounts of the suspected food under medical supervision, in an open or blinded manner.[81] Although double-blind, placebo-controlled challenges are least prone to bias[84] and therefore considered the 'gold standard' diagnostic test for food allergy, they are labour-intensive, time-consuming and may induce anaphylaxis.[85] They are particularly difficult to perform in large epidemiological studies.[90] Therefore, our knowledge surrounding the relationship between food allergy and other conditions stems mainly from studies which have utilised alternative criteria to define food allergy.[80]

1.2.2.2 The Relationship between Food Allergy and Asthma

Numerous studies have demonstrated an association between allergic sensitisation to food allergens and asthma. Within the MAS cohort, for example, children sensitised to hen's egg, cow's milk, soy or wheat (specific IgE to ≥ 0.35 kU/l) at 1 and 2 years of age, had a 10.6 times higher risk of developing asthma than children never sensitised to any of these food allergens.[91] Meanwhile, Wang et al. found that amongst 504 children aged 4-9 years enrolled in the National Cooperative Inner City Asthma Study (NCICAS), 45% had evidence of sensitisation to at least one of the six most common food allergens.[92] This is much higher than the estimated prevalence of positive specific IgE to at least one food across Europe, which is 10.1% overall and 2.7% in children aged 0-17 years.[82]

Studies taking clinical symptoms and sensitisation into account, also support an association between food allergy and wheezing disorders. Schroeder et al., for example, studied 296 children who were less than 6 years and 271 children aged 6 years or older enrolled in a family based food allergy study. Symptomatic food allergy (defined as typical allergic symptoms within 2 hours of ingestion of a food and either an IgE ≥ 0.1 kU/l or a positive skin prick test to that food) was strongly associated with parentally reported physician diagnosed asthma in both the younger (OR 5.3, 95% CI 1.7-16.2) and older children (OR 4.9, 95% CI 2.5-9.5). Furthermore, the risk of asthma was highest in those with symptoms suggestive of severe food allergy and those with two or more allergies.[93] The Urban Environment and Childhood Asthma (URECA) study offered further insights into the relationship between food allery and early childhood wheeze. This was a prospective, inner-city birth cohort, established in 2005-2007 to study the effects of specific urban exposures on the development of recurrent wheeze and asthma. From birth to 5 years, parents were asked annually about symptoms suggestive of food allergy and at 1,2,3 and 5 years levels of specific IgE to milk, egg and peanut were measured. Children with food allergy were more likely to wheeze from the third year of life onwards than those without food allergy (OR 3.9, 95% CI 1.7-5.7, $p<0.001$ for wheeze in year 4). However, no association between food allergy and wheeze in the first two years of life was observed. In the URECA study, food allergy was defined as a positive IgE level (≥ 0.35 kU/l) to milk, egg and/or peanut and a physician diagnosis of food allergy or parental report of a previous reaction suggestive of food allergy.[94] It is therefore likely that in this and the aforementioned study by Schroeder et al., cases of food allergy were overestimated.

Recently, Saarinen et al. prospectively followed 118 children with challenge proven cow's milk allergy who were enrolled in a population-based cohort study. Compared to a control group, those with IgE-positive challenge proven cow's milk allergy in the first year of life, were more likely to have asthma (31 vs 13%, p <0.01), rhinoconjunctivitis (66 vs 21%, p <0.01) and atopic eczema (81 vs 26%, p <0.01) at school age.[95] The same study group also demonstrated higher eNO levels (mean log eNO 1.14, 95% CI 1.08-1.19 vs mean log eNO 1.02, 95% CI 0.97-1.07) and increased bronchial hyperresponsiveness at school age among those with cow's milk allergy. [96]

Food allergy is associated not only with an increased risk of developing asthma but also with worse asthma outcomes. Wang et al., for example, demonstrated that children in the NCICAS cohort who were sensitised to one more foods had higher rates of asthma hospitalisation (p= 0.001) and higher rates of steroid use (p=0.025) than non-sensitised individuals.[92] Furthermore, Roberts et al. demonstrated that children with co-existing asthma and food allergy are at increased risk of near fatal asthma. When 19 children ventilated for an exacerbation of asthma were compared with 38 age-matched controls who had attended hospital with a non-life-threatening exacerbation of asthma, the odds ratio for food allergy among cases was 8.58 (95% CI 1.85-39.71).[97]

The association between food allergy and asthma may just be statistical given that both are linked to atopy. However, there may be a casual pathophysiological pathway with clinical implications.[83] Improving our understanding of the association between the two conditions is important because if food allergy is a predictor of subsequent asthma, early intervention or prevention may be possible. [80] Further population-based cohort studies utilising oral food challenges are therefore needed.

1.3 Asthma

Asthma is a chronic condition which affects all age groups. There are multiple phenotypes, which vary according to the age of onset, symptoms, exacerbating factors, response to treatment and severity.[33]

1.3.1 Severe Asthma

Although patients with severe asthma represent only 10% of all asthma patients, they account for 50% of healthcare costs[7] and have high morbidity and mortality.[98] According to an ERS/ATS Taskforce, severe asthma is asthma requiring treatment with high dose inhaled corticosteroids and a second controller for the previous year or systemic corticosteroids for more than 50% of the previous year to maintain control, or asthma which remains uncontrolled despite this therapy.[99] Features of poor control include an ACQ score persistently greater than 1.5 or an ACT score less than 20, frequent severe exacerbations and airflow limitation (FEV_1 <80%). Severe asthma is a complex disease with a wide variety of pathophysiological mechanisms, clinical features and outcomes. To improve understanding and management of severe asthma, attempts have been made to identify asthma phenotypes, using hypothesis-based and unbiased approaches.[99,100] A phenotype is defined as the composite, observable characteristics of an organism, resulting from interaction between its genetic make-up and environmental influences.[99] To date, most asthma phenotypes identified have been characterised by clinical features rather than factors which provide insights into underlying disease pathology.[100]

Fitzpatrick et al. identified four clusters of children with asthma from 161 children enrolled in the Severe Asthma Research Program (SARP): 1) later-onset asthma with normal lung function, 2) early-onset atopic asthma with normal lung function, 3) early-onset atopic asthma with mild airflow limitation and 4) early-onset atopic asthma with advanced airflow limitation.[101] These clusters were derived by applying cluster analysis to 12 variables covering demographics, asthma symptoms, medication and health care use, eNO and atopic sensitisation. Children with severe asthma were present in all four clusters with no one cluster corresponding to proposed definitions of severe asthma. This highlights the heterogeneity of severe asthma and need to ultilise unbiased approaches to define phenotypes. Furthermore, the clusters were distinct from those previously identified in adults, suggesting that findings from adults with severe asthma cannot be translated directly to children. The SARP clusters were replicated in 611 children from three Childhood Asthma Research and Education (CARE) Network clinical trials and their associations with treatment responses were explored.[102] Based on the finding that an early onset, severe lung function cluster responded best to fluticasone/salmeterol and an early onset, mild

airflow limitation cluster (with greatest comorbidity) showed limited treatment responses, it has been suggested that identifying asthma phenotypes may enable clinicians to personalise treatment regimes. This theory is supported by Howrylak et al., who divided 1041 children enrolled in the Childhood Asthma Management Program (CAMP) into five distinct clusters.[103] CAMP was a multi-centre trial of 1041 children aged 5 to 12 years with mild to moderate asthma who were randomly assigned to receive budesonide, nedocromil or placebo (short acting beta-agonist as required). Responses to inhaled corticosteroids differed between patients in the two most severe clusters. Patients in one cluster (low atopic burden, worst lung function) responded well to budesonide and nedocromil compared to placebo, whereas in the other cluster (high atopic burden, low lung function), neither budesonide nor nedocromil reduced the rate of exacerbations.

However, other studies have suggested that phenotyping asthma using clinical variables may be of limited clinical benefit. The TENOR study group identified five clusters of children (aged 6-11 years) and adolescents/adults (aged ≥ 12 years) with difficult-to-treat asthma and related these to health outcomes including exacerbations, asthma control and quality of life.[104] The clusters were distinguished by gender, atopic status and race in both age groups and additionally, by passive smoke exposure in children and aspirin sensitivity in adolescents/adults. The aspirin sensitive cluster (which included mainly white, female patients with late-onset asthma and atopy) experienced more exacerbations and had a poorer quality of life. Despite robust differences between the paediatric clusters, these were not related to subsequent health outcomes. Meanwhile, in a longitudinal cohort of 112 adult patients with severe asthma, Bourdin et al. identified five clusters using a SARP algorithm.[105] These differed in terms of age, asthma duration, lung function, blood eosinophil levels, ACQ-6 scores and diabetes comorbidity. However, all the cohorts shared similar outcomes, including ACQ-6 score, exacerbation rate and treatment requirements.

It is hoped that with the emergence of new biomarkers including omics technologies novel asthma phenotypes will be discovered. Omics technologies, which include genomics, proteomics, lipidomics and metabolomics explore the roles and relationships of molecules within biological systems. They involve large scale surveys, making no a priori assumptions about which components may be associated with a particular disease. Phenotypes integrating clinical data and omics may enable a more personalised approach to the treatment of patients with severe asthma.[11]

1.3.2 Asthma Exacerbations

Asthma exacerbations are a cause of substantial morbidity, including poor quality of life and accelerated declines in lung function.[106-109] They are also an important risk factor for asthma death.[110] Therefore, one of the main goals of asthma management is to prevent exacerbations.[12] Identifying patients at high risk for asthma exacerbations could lead to targeted treatments and reduced morbidity.[111]

1.3.2.1 Definitions

Exacerbation incidence and severity are dependent on the definitions used for exacerbations.[12] If standardised defintions are not used in clinical trials, data from these cannot easily be compared or pooled for meta-analyses. Therefore, in 2009 the American Thoracic Society (ATS)/European Respiratory Society (ERS) published a consensus statement, which included the following definitions for asthma exacerbations.

Severe asthma exacerations were defined as asthma exacerbations requiring use of systemic corticosteroids (oral or parenteral) or an increase from a stable maintenance dose for at least 3 days or an asthma-related hospitalisation or visit to the emergency department requiring oral corticosteroids.

Moderate exacerbations were defined as a deterioration in symptoms, a deterioration in lung function and/or an increase in bronchodilator use for at least 2 days, but not severe enough to require systemic corticosteroids or hospitalisation.[112]

1.3.2.2 Risk Factors for Exacerbations

In order to prevent asthma exacerbations and their consequences, those at risk need to be identified so that targeted treatments can be developed.[111,113] Although asthma exacerbations are more common in those with severe disease, they occur across all levels of disease severity.[113] It has therefore been been hypothesised that patients with frequent exacerbations may represent a separate phenotype of disease with potentially unique pathogenic mechanisms.[106,114]

In support of this theory, studies in both children and adults have consistently shown that previous asthma exacerbations are the best predictor of subsequent exacerbations, regardless of disease severity.[111,115] Miller et al., for example, conducted a prospective analysis of 2780 patients aged ≥ 12 years enrolled in the TENOR study. They found that patients with a severe exacerbation in the previous 3 months were over six times more likely to experience future exacerbations compared to those without a recent exacerbation. This association remained after adjustment for asthma severity using three different methods of severity assessment.[116] Similarly,

amongst children aged 6 to 11 years with severe/difficult to treat asthma enrolled in the TENOR study, future severe exacerbations over 6 months were most strongly predicted by one or more severe exacerbations in the preceding 3 months (OR 3.08, 95% CI 2.21-4.28). [117] An association between previous and future exacerbations has also been demonstrated in a large population population based study conducted by Bloom et al.[118] They used electronic health care records to identify 51,463 adults aged 18-55 years with asthma who had at least 7 years of follow up data. 36% of patients had one or more exacerbations during follow up. The odds of having a future exacerbation were significantly higher for patients who had experienced a previous exacerbation, particularly if an exacerbation had occurred recently. For example, the odds ratio of having a future exacerbation was 6.7 (95% CI 6.1-7.4) if an exacerbation had occurred in the past year, compared to 2.6 (95% CI 2.4-2.8) if an exacerbation had occurred five years previously. Furthermore, the likelihood of a future exacerbation was higher in patients with a history of multiple exacerbations and in patients with a past history of a severe exacerbation.

Poor asthma control is also a recognised risk factor for asthma exacerbations in children and adults.[119] [120] The Asthma Control Test (ACT) comprises five questions and produces a score ranging from 0 to 25, where higher scores indicate better disease control.[121] In post-hoc analysis of data from a 12-month prospective cohort study involving asthma patients aged 15-60 years, Wei et al. found that lower Asthma Control Test (ACT) scores at baseline in those with uncontrolled and partly controlled asthma were associated with an increased risk of future asthma exacerbations (OR 3.65, 95% CI 2.20-6.04 and 5.75, 95% CI 2.91-11.38, respectively).[122] Meanwhile, in the TENOR study, Hasselkorn et al. found that in addition to recent severe exacerbations, poorly controlled asthma (according to the impairment component of the National Heart, Lung and Blood Institute guidelines) significantly predicted future severe exacerbations (OR 1.59, 95% CI 1.14-2.23).[117] In another study, the ability of the Children's Asthma Control Test (C-ACT) and spirometry to predict asthma exacerbations in children aged 4 to 11 years were evaluated. 32 out of 97 patients (33%) had one or more asthma exacerbations during a 6-month period of follow up. Baseline C-ACT was significantly lower (indicating poorer asthma control) among patients with asthma exacerbations than those without (22.9 vs 24.5, p=0.015) and in logistic regression the occurrence of an exacerbation was inversely associated with C-ACT (β=-0.023, p= .042). Spirometric values, including FEV_1 % predicted FEV_1/FVC ratio, FEF_{25-75} and PEF, were not, however, associated with asthma exacerbations (p>0.5 in all cases).[123]

In other studies of both adult and children, an association between reduced lung function and asthma exacerbations has been demonstrated. Using data from the Childhood Asthma Management Program, Fuhlbrigge et al. examined the relationship between prebronchodilator FEV_1 and asthma related events in 417 children with mild to moderate asthma (assigned to

placebo). Compared to children with an $FEV_1 \geq 100\%$ predicted, children with an FEV_1 of 80 to 99%, 60 to 79% and <60% were 1.3, 1.8 and 4.8 times more likely to have a serious exacerbation over a 4-month period.[124] Meanwhile, amongst participants from all of the CAMP treatment groups Wu et al. identified a lower FEV_1/FVC ratio as an idependent risk factor for severe exacerbations (β-estimate -0.023, 95% CI -0.040 to -0.0061, p=0.0076).[125] Given that FEV_1 is often normal in children with asthma, Rao et al. investigated the relationship between FEF_{25-75} and asthma morbidity in the setting of a normal FEV_1. Spirometry results were obtained in 744 children aged 10-18 years diagnosed with asthma over a 10-year period at a tertiary children's hospital. Medical records in the one year prior to and following spirometry were reviewed for details on asthma severity and outcomes. Children with a low FEF_{25-75} and low FEV_1/FVC but normal FEV_1 were 6 times more likely to experience exacerbations than than those with normal spirometry (OR 6.3, 95% CI 1.86-33.42., p<0.001).[126]

Other risk factors for asthma exacerbations appear to differ between children and adults.[115,127,128] In children, for example, younger age is a predictor of exacerbations whereas in adults the opposite is true.[115,118] Mahut et al. reviewed asthma control and exacerbations over the past 3 months in 359 children receiving inhaled corticosteroids for persistent asthma. In a multivariate logistic regression model, with severe exacerbations as the dependent variable and age, season, long acting bronchodilator administration and inhaled corticosteroid (ICS) dose as explanatory variables, age, season and ICS dose significantly influenced the risk of a severe exacerbation. For each year increase in age from infancy to adolesence, the risk of a severe exacerbation decreased by 15%.[129] Similarly, Wu et al. found that increasing age reduced the risk of exacerbations amongst children enrolled in the Childhood Asthma Management Program (β-estimate -0.10, 95% CI -0.16 to -0.052, p<0.001).[125] Regarding gender, boys are more likely to suffer from asthma exacerbations than girls before puberty. Thereafter, however, the sex difference reverses and females have a greater incidence and prevalence of exacerbations than males.[130] In adults, co-morbidities including obesity, depression and gastro-oesophageal reflux disease have been linked to asthma exacerbations, whereas in children co-morbid allergic disease may be important.[115,128]

In the Childhood Asthma Management Program, Wu et al. found no association between the number of positive skin prick tests at baseline and asthma exacerbations. However, cluster analysis of the CAMP participants by Howrylak et al. suggested that atopy is more common amongst patients who experience frequent exacerbations.[103] Other studies have also suggested this. Ortega et al., for example, applied a cluster analysis approach to a sample of 2205 adults and 2435 children with asthma. Seven adult and six paediatric clusters were identified. The rate ratio for having an asthma exacerbation was significantly higher in cluster 7 of the adult participants (RR 2.88, 95% CI 2.46-3.36), which was characterised by female patients with severe asthma, a

lower asthma control score, gastro-oesophageal reflux disease and skin allergies. Meanwhile, the paediatric cluster with the highest rate of exaerbations (RR 2.36, 95% CI 2.11-2.64) was characterised by patients with severe asthma, a lower asthma control score and sinus and skin allergies.[131] Similarly, Just et al. applied cluster analysis to 19 variables from 315 children enrolled in the Trosseau Asthma Program in France. Three clusters were identified, one of which was characterised by a higher number of positive skin prick tests to inhaled and food allergens, high levels of uncontrolled asthma and high rates of exacerbations requiring hospitalisation.[132] Finally, when Lazic et al. used a machine learning approach to cluster children within two population-based birth cohorts into different classes of atopic sensitisation, one of the five clusters identified (the cluster with sensitivity to a wide variety of allergens) was associated with a higher risk of asthma exacerbations.[71] Further studies utilising component resolved diagnostics would help to clarify the relationship between allergy and asthma exacerbations.

1.4 Summary of Knowledge Gaps

1.4.1 Early Childhood Wheeze

The International Study of Asthma and Allergies in Childhood (ISAAC)[22] and European Community Respiratory Health Survey (ECRHS)[23] have previously demonstrated that prevalence rates of asthma in school aged children and adults vary considerably between countries. However, studies comparing prevalence rates of early childhood wheeze between countries are lacking. Such studies are needed to help improve our understanding of the aetiology of early childhood wheeze.

Birth cohort studies have identified a number of important risk factors for early childhood wheeze, including respiratory tract infections (particularly those due to RSV and rhinoviruses), day care attendance, older siblings, environmental tobacco smoke exposure and male gender.[9] Studies have also demonstrated that breastfeeding is protective.[51-53] However, studies looking at whether the timing of complementary feeding influences the risk of wheeze have shown conflicting results.[56,57] Given that infant feeding practices are modifiable, further research is needed to determine their role in the aetiology of early childhood wheeze. Evidence regarding the relationship between early onset food allergy and early childhood wheeze is also limited. Food allergy is known to be associated with an increased risk of developing asthma and worse asthma outcomes.[92,97] However, few studies have explored the relationship between food allergy and wheezing disorders in early childhood and of those that have, none have utilised food challenges to diagnose food allergy. If food allergy is associated with an increased risk of early childhood wheeze, strategies to prevent food allergy may also help to reduce the burden of early childhood wheeze. Therefore, studies aimed at improving our understanding of the relationship between challenge proven food allergy and early childhood wheeze are needed.

1.4.2 Allergy and Asthma

It has long been recognised that allergy and asthma are closely linked, particularly in children. Furthermore, allergic diseases and allergic sensitisation appear to be associated with severe asthma. For example, children with food allergy have higher rates of hospitalisation, systemic corticosteroid use and mechanical ventilation than those without.[90,97] Meanwhile, increasing levels of specific IgE and increasing skin prick test wheal diameters to aeroallergens are associated with markers of asthma severity including poor lung function.[67-70,133] However, no studies comparing prevalence rates of allergic diseases and allergic sensitisation in patients with mild to moderate and severe wheeze/asthma across the life course have been undertaken. Such studies would be beneficial as an improved understanding of how allergy influences asthma severity across the life course could lead to improvements in wheeze/asthma management.

1.4.3 Asthma Exacerbations

Previous research has identified that a history of previous asthma exacerbations and poor asthma control are the best predictors of future asthma exacerbations in adults and children.[111,115] However, given that exacerbations may occur in non-symptomatic patients and those with no history of exacerbations, further research is needed to identify novel risk factors which account for the fact that asthma is a heterogeneous disease.[98,111] In recent years, attempts have been made to identify asthma phenotypes. However, there is limited evidence that these reliably predict clinical outcomes. Indeed, studies looking at the relationship between asthma phenotypes and future exacerbation rates have shown conflicting results.[104,105,131,132] The relationship between atopy and asthma exacerbations is also inconclusive.[134] Recent research in children, for example, suggests that specific patterns of atopic sensitisation are more strongly associataed with an increased risk of asthma exacerbations than conventional atopy.[71] Further studies are therefore needed to clarify whether clinical phenotypes and patterns of atopic sensitisation can predict asthma future exacerbations across the life course.

1.5 Overview of Thesis

This thesis uses data collected as part of the EuroPrevall birth cohort study and the Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (UBIOPRED) study to provide new inights into the aetiology of early childhood wheeze, the role of allergic disease and allergic sensitisation in asthma across the life course and risk factors for exacerbations in patients with severe asthma/preschool wheeze.

1.5.1 Early Childhood Wheeze

The EuroPrevall birth cohort study was established in 2005 to examine prevalence patterns of food allergies in children across Europe.[135] Children from nine European countries were recruited at birth and were routinely followed up at 12 and 24 months using standardised questionnaires. Those with signs and symptoms suggestive of possible food allergy underwent additional assessments skin prick testing and measurement of specific IgE with or without a double-blind, placebo-controlled food challenges. The 12 and 24-month questionnaires included multiple questions relating to wheeze. Detailed information on participants' birth history, family history, maternal diet, environmental exposures (including cigarette smoke and pets), dietary intake during the first two years of life and other medical problems was also collected. Therefore, the EuroPrevall birth cohort is an ideal cohort in which to examine the prevalence of and risk factors for early childhood wheeze.

Birth cohort studies, in general, are the ideal study design for evaluating risk factors for disease that begin in early childhood.[135] Their prospective nature means that the temporal relationship between environmental exposures and the onset of the disease can be determined. Collecting data at multiple time points (starting antenatally) also means that relevant time windows of exposure and the effects of cumulative exposure can be studied. Furthermore, they allow interactions between different exposures and genetic factors to be studied.[31 136]

The EuroPrevall birth cohort is the largest birth cohort to date to examine risk factors for early childhood wheeze. It is also the first to evaluate variations in the prevalence of preschool wheeze across different countries. The International Study of Asthma and Allergies in Childhood (ISAAC) and European Community Health Respiratory Survey (ECHRS) compared the prevalence of asthma symptoms including wheeze between countries worldwide. However, neither of these studies included preschool children. One study has examined variations in prevalence rates of wheeze at 4 years of age across Europe. However, this utilised data from 10 individual MeDALL (Mechanisms of the Development of ALLergy) cohorts.[29] Comparing data from individual studies is not ideal due to use of different defintions and methods. By comparing the prevalence of early childhood

wheeze across Europe in the EuroPrevall cohort, it may be possible to identify new risk factors and determine the importance of those which are already known. Furthermore, the EuroPrevall cohort provides a unique opportunity to explore the association between challenge proven food allergy and early childhood wheeze.

1.5.2 Allergy and Asthma

The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (UBIOPRED) study is a multi-centre, prospective observational cohort study of preschool wheeze and asthma, which was established in 2009. Participants with mild to moderate and severe disease were recruited into adult, school and preschool age cohorts. All participants underwent a baseline visit at which a detailed asthma and allergic disease history was taken. Skin prick testing, specific IgE measurement and component resolved allergen diagnostics using the ISAC Chip® (ThermoFisher Scientific, Uppsala, Sweden) were also performed.

An association between allergic disease and asthma has long been recognised. However, most studies demonstrating this have focused on children or young adults and have defined asthma on the basis of a doctor's diagnosis of asthma or a history of wheeze without objective evidence of bronchoconstriction, airway inflammation or airway hyperresponsiveness. [65,66] It has been demonstrated that in epidemiological studies both children and adults may be incorrectly defined as 'cases' if case definitions are based on parentally reported wheeze or a self reported general practitioner (GP) diagnosis of asthma.[59]

In the UBIOPRED study, comprehensive diagnostic criteria were used to ensure that diagnoses of asthma and preschool wheeze were accurate. Participants in the severe cohorts, for example, had to have been under tertiary follow up for at least six months to exclude alternative diagnoses and in the school age and adult cohorts, lung function testing ± bronchodilator reversibility and methacholine challenge testing were performed. UBIOPRED is also the first study to recruit preschool wheeze cohorts on the basis of a consensus definition. All of the UBIOPRED cohorts were assessed in the same way, allowing the prevalence of allergic disease and allergic sensitisation in patients with asthma/preschool wheeze to be assessed across the life course. In this analysis, differences between patients with mild to moderate and severe asthma/preschool wheeze will also be assessed to provide a detailed insight into the role of allergic disease and allergic sensitisation in asthma/preschool wheeze.

1.5.3 Asthma Exacerbations

The severe UBIOPRED cohorts were followed up longitudinally (after 12-18 months). At follow up details of asthma/wheeze exacerbations were recorded. Asthma exacerbations are associated with considerable patient morbidity and place a major burden on healthcare resources. Therefore, the ability to predict and potentially prevent asthma exacerbations would be beneficial at both the individual patient level and from a public health point of view.[12 115] According to the EACCI Postion Statement on asthma exacerbations and severe asthma, further studies with improved designs are needed to properly characterise the risk of asthma exacerbations.[98] UBIOPRED will address this need by exploring risk factors for asthma exacerbations across the life course. Previous research suggests that asthma and atopy encompass a number of different phenotypes which differ in their association with clinical outcomes such as asthma exacerbations.[59] This analysis will focus on determining whether clinical clusters and ISAC component atopy clusters are associated with future asthma exacerbations in patients with severe asthma. A wide range of other clinical characteristics will also be assessed as potential risk factors. In previous studies looking at the association between atopy/allergic disease and asthma exacerbations, sensitisation has been determined according to the results of skin prick testing and measurement of specific IgE to selected panels of food and inhalant allergens. By using ISAC Chip® data to cluster participants, this analysis aims to provide new insights into the relationship between specific patterns of allergic sensitisation and asthma exacerbations. Inclusion of preschool children, school age children and adults in the UBIOPRED study also means that risk factors for asthma exacerbations can be assessed across the life course. Evidence supporting risk prediction of exacerbations in preschool children is more limited than in other age groups.[115] Hence, inclusion of this age group is particularly important.

1.6 Aims, Objectives and Hypotheses

1.6.1 Early Childhood Wheeze- Prevalence Patterns and Risk Factors

The aims of this work were :

- To determine the prevalence of early childhood wheeze across Europe.
- To evaluate risk factors for wheeze and how these differ across Europe.

Evaluation of risk factors focused on food allergy, infant feeding and smoke exposure. Given that food allergy is one of the first manifestiations of atopy, it was hypothesised food allergy increases the risk of early childhood wheeze.

It was also hypothesised that the following are risk factors for early childhood wheeze:

- Shorter duration of breastfeeding.
- Decreased overlap between breastfeeding and solids.
- Later introduction of solids.
- Maternal smoking during pregnancy and early childhood.
- Other household smokers.
- Low birth weight, short length and low gestation.

1.6.2 Allergic Sensitisation and Allergic Disease in the UBIOPRED Cohorts

The overall aim of this work was to assess whether the prevalences of allergic sensitisation and allergic disease explain the differences between mild to moderate and severe asthma/preschool wheeze across the life course.

Specific objectives were:

- To compare the prevalence of eczema, allergic rhinitis and hay fever in adults, school aged children and preschool children with severe verses mild to moderate asthma/preschool wheeze.
- To compare the prevalence of food allergy (defined on the basis of clinical symptoms and skin prick testing/specific IgE results) in adults, school aged children and preschool children with severe verses mild to moderate asthma/preschool wheeze.
- To compare the prevalence of allergic sensitisation and atopy in adults, school aged children and preschool children with mild to moderate verses severe asthma/preschool wheeze.

The following hypotheses were tested:

- The prevalence of allergic diseases and allergic sensitisation differs across the life course in patients with asthma/preschool wheeze.
- The prevalence of allergic diseases and allergic sensitisation is higher in patients with more severe asthma/preschool wheeze.

1.6.3 Asthma Exacerbations in the UBIOPRED Cohorts

The aims of this work were:

- To assess whether rates of future exacerbations differ between clinical clusters of patients with severe asthma/preschool wheeze.
- To assess whether rates of future exacerbations differ between patients with different patterns of allergic sensitisation.
- To evaluate whether specific clinical characteristics are associated with more frequent severe asthma exacerbations. Characteristics explored included demographic factors, previous asthma history, co-morbidities, allergic sensitisation, environmental exposures, reported triggers for respiratory symptoms, clinical cluster assignment and ISAC component atopy cluster assignment.

The following hypotheses were tested:

- Rates of future exacerbations differ between clinical wheeze/asthma clusters.
- Rates of future exacerbations differ according to patterns of allergic sensitisation.

Chapter 2: EuroPrevall Methods

This thesis uses data collected as part of the EuroPrevall birth cohort study. The methodology of this has previously been described in the following papers:

Keil T, McBride D, Grimshaw K, Niggeman B, Xepapadaki P, Zannikos K et al. The multinational birth cohort of EuroPrevall: background, aims and methods. *Allergy* 2010; 65: 482-490.[135]

McBride D, Keil T, Grabenhenrich L, Dubakiene R, Drasutiene G, Fiocchi A, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Pediatr Allergy Immunol* 2012; 23: 230-9.[137]

Aspects of the methodology that are relevant to this thesis have been summarised below.

2.1 Study Design

The EuroPrevall project was launched in 2005 to address knowledge gaps in food allergy. It was funded by the European Union and involved over 60 partners, including patient organisations, the food industry and research institutions from Europe, Ghana, Russia, China and India.[135,137] As part of the EuroPrevall project, a birth cohort was established across nine European countries (chosen to ensure that different climatic and cultural regions were represented).[135] Prospective birth cohort studies are the best study design for evaluating risk factors for diseases that present in early childhood.[135] Collecting data at repeated time points (starting in pregnancy) means that the temporal relationship between environmental exposures and disease onset can be determined.[31] The EuroPrevall birth cohort study was primarily established to examine variations in the prevalence of food allergies across Europe.[135] However, it also provides a unique opportunity to assess the prevalence of early childhood wheeze across Europe and evaluate potential risk factors.

Longitudinal, prospective evaluation of the EuroPrevall cohort began at birth and included routine follow up of all participants at 12 and 24 months using standardised questionnaires. Additional assessments including skin prick testing, measurement of specific IgE levels with or without a double-blind, placebo-controlled food challenge (DBPCFC) were undertaken according to a standardised protocol whenever parents reported signs or symptoms suggestive of food allergy in their children.[135]

2.2 Study Population

Parents and their children were recruited ante- and postnatally from nine study centres between October 2005 and February 2010.[137] Study centres included Reykjavik (Iceland), Southampton (The United Kingdom), Amsterdam (The Netherlands), Berlin (Germany), Lodz (Poland), Vilnius (Lithuania), Madrid (Spain), Milan (Italy) and Athens (Greece).[135]

Inclusion criteria were a gestational age of at least 34 weeks and a good condition at birth (defined as an Apgar score of at least 7 at 5 minutes of life). Families that were unable to give informed consent (due to language or communication difficulties) and infants participating in other studies examining atopy or allergic disease were excluded. Each centre obtained approval for the study from their local ethics committee and written informed consent was obtained from all parents.[135,137]

2.3 Data Collection

2.3.1 Questionnaires

Questionnaires were chosen as the main data collection tool. These are a cost effective and efficient means of collecting large amounts of information from a large sample of people.[138] Use of standardised questions also means that data collected at different study centres can be compared. A potential limitation of questionnaires is that questions may be misinterpreted by respondents, particularly if the language of the questionnaire is not appropriate to the population being studied. Furthermore, if closed questions with a limited number of responses are used, important information may not be captured.[138] The EuroPrevall questionnaires (Appendices A.2 and A.3) were based mainly upon those used in previous epidemiological studies such as the International Study of Asthma and Allergies in Childhood (ISAAC) and the Multicentre Allergy Study (MAS) with use of study specific questions where necessary.[135] The ISAAC questionnaires have previously been validated in many languages for assessing wheeze in school age children.[139] Furthermore, all questionnaires were translated into different languages and verified with back-translation into English to limit the potential for misunderstanding. They were conducted via telephone or in person by trained interviewers.

At recruitment, data were collected on birth details, maternal diet, family history (including allergic conditions), socio-demographic status and environmental exposures, including cigarette smoke and pet ownership. At follow up time points, data were collected on signs and symptoms of allergic disease including wheeze, the child's feeding history, exposure to cigarette smoke, infections and day care attendance.[135]

2.3.2 Identification of Children Requiring Further Evaluation

Parents were asked to contact their local study team if their child had any signs or symptoms potentially related to food ingestion e.g. eczema, urticaria, gastrointestinal symptoms or wheezing. A standardised protocol was subsequently used to determine which children required further assessment. Symptomatic children were invited to the study centre for a physical examination, including completion of the SCORing Atopic Dermatitis (SCORAD) tool for assessing the severity and extent of eczema, skin prick testing and venepuncture (to allow measurement of specific IgE levels). Parents were also asked to complete a symptomatic questionnaire containing similar questions to the 12 and 24-month questionnaires.

According to international guidelines, a detailed clinical history is the most important step in the diagnosis of food allergy with skin prick testing and measurement of specific IgE providing supporting information.[81] Studies have suggested that these have good sensitivity but poor specificity.[85] This means that in the absence of a suggestive clinical history, a negative skin prick test or specific IgE makes a diagnosis of food allergy unlikely. However, negative results need to be interpreted with caution in the presence of a suggestive clinical history particularly as these are expected in non-IgE mediated food allergy. Furthermore, using the traditional cut-offs of 3mm and 0.35 kU/l, respectively, skin prick testing and specific IgE measurement are associated with high false positive rates. Therefore, a positive test alone does not necessarily predict clinically relevant food allergy. To ensure accurate diagnoses of food allergy children meeting one or more of the following criteria were invited for a double-blind, placebo-controlled food challenge (DBPCFC):

- Elevated allergen-specific serum IgE (>0.35 kU/l) and not eating the food regularly without clinical signs or symptoms.
- Positive skin prick test (≥3mm wheal diameter) and not eating the food regularly without signs or symptoms.
- Immediate (within 2 hours) objective clinical signs or symptoms after ingestion of a single food.

- Repetitive (on at least 2 occasions) subjective clinical signs or symptoms after ingestion of a single food.
- Clear improvement or absence of clinical signs or symptoms e.g. eczema or diarrhoea following an elimination diet.

2.3.3 Skin Prick Testing

Skin prick tests were undertaken using ALK (ALK-Abello, Horsholm, Denmark) allergen solutions with histamine and saline as positive and negative controls, respectively. One drop of each solution was applied to the forearm and pricked with a 1-mm single-headed lancet (ALK-Abello, Horsholm, Denmark). Results were read after 15 minutes. A positive result was defined as a wheal diameter of 3mm or more.

2.3.4 Measurement of Food Specific IgE

Where applicable, serum was screened for the six most common food allergens (cow's milk, hen's egg, soy, wheat, fish and peanut) using the Phadia fx5 screening test (Phadia diagnostics, Uppsala, Sweden). If screening was positive, the serum was tested for specific IgE antibodies to these foods. All measurements were performed at the allergy laboratory of the Department of Paediatric Pneumonology and Immunology, Charité University Medical Centre, Berlin, Germany.

2.3.5 Double-Blind, Placebo-Controlled Food Challenges (DBPCFCs)

Challenges were performed under the supervision of a trained paediatrician according to a standardised protocol. They consisted of nine steps with increasing doses given every 20 minutes. Allergenic foods were masked in extensively hydrolysed or amino acid formula for bottle fed infants or vanilla-orange pudding for older infants. Children with more than one possible food allergy were challenged with at least a 1:2 ratio of placebo to active food in randomised order. Active and placebo challenge days were separated by at least 48 hours.

A positive challenge was defined as objective symptoms such as urticaria or angioedema within 2 hours of the final dose or worsening eczema with an increase in SCORAD ≥10 within 48 hours of starting the challenge.[140] Food allergy was diagnosed without a DBPCFC in children with a clear history of anaphylaxis and elevated allergen-specific serum IgE.

2.4 Statistical Analysis

2.4.1 Generating the Dataset for this Analysis

The EuroPrevall birth cohort database is held at the Charité University Medical Centre, Berlin, Germany. This includes:

- A baseline dataset containing data from:
    - EuroPrevall Baseline Questionnaire Form 1: Birth Data.
    - EuroPrevall Baseline Questionnaire Form 2: Questions for the mother.
- Datasets containing data from:
    - EuroPrevall Baseline Questionnaire Form 3: Allergy history of the mother.
    - EuroPrevall Baseline Questionnaire Form 4: Allergy history of the father.
- A 12-month questionnaire dataset containing data from 12-Month Follow up Questionnaires *(*Appendix A.2) and Symptomatic Visit Questionnaires.
- A 24-month questionnaire dataset containing data from 24-Month Questionnaires and Symptomatic Questionnaires for those aged 13-24 months (Appendix A.3).
- A dataset containing food challenge outcome data.
- A dataset containing feeding variables generated using follow-up data.

In symptomatic children and controls, the same data were collected at multiple time points (due to the completion of symptomatic and annual questionnaires). Annual questionnaires were not, however, always completed at 12 and 24 months. In order to determine which questionnaires to use for this analysis (aiming to include those completed as close to 12 and 24 months as possible), the ages of all participants at times of data collection were calculated. This revealed overlap between the 12-month questionnaire and 24-month questionnaire datasets i.e. some questionnaires within the the '12-month' dataset had been completed closer to 24 months than 12 months. Given that this analysis relied upon data on wheeze at specific time points, the following approach was taken:

- The original 12 and 24-month questionnaire datasets were merged.
- Any questionnaires completed before 6 months or after 30 months were excluded.
- Any questionnaire data obtained between the ages of 6 and less than 18 months of age were labelled as one-year data and any questionnaires data obtained between the ages of 18 and 30 months were labelled as two-year data.

- If two questionnaires were completed within the same time period (due to completion of a symptomatic and an annual questionnaire), the questionnaire completed closet to 12 months or 24 months was utilised.
- New 12-month and 24-month datasets containing only one entry per participant were generated.

These new datasets were subsequently merged with the baseline dataset, data on parental allergies, the food challenge outcome data and the infant feeding data. All subsequent analyses were undertaken in STATA SE 13 (StataCrop, College Station, USA).

It was also necessary to transform non-dichotomous catriegorical variables into dichotomous variables and generate a number of new variables. Variables not required for this analysis were removed from the dataset. A comprehensive literature search was undertaken to identify variables which may be important risk factors for wheeze. The findings of the literature search have previously been discussed in the introduction to this thesis.

2.4.2 Exposures

2.4.2.1 Dichotomous Variables

Table 1 describes how dichotomous variables were generated using data collected at baseline and follow up time points.

Table 1 Description of Dichotomous Variables Generated using Questionnaire Data

Variable	Source of data	Question/questions asked	Dichotomous classification

Form of delivery	Baseline questionnaire	• Form of delivery
		  ○ Normal, unassisted
		  ○ Forceps, vacuum assisted
		  ○ Vacuum extraction
		  ○ Caesarean section, planned
		  ○ Caesarean section, emergency
			Children were grouped according to whether they were born via Caesarean section or vaginally.

Parental ethnicity	Baseline questionna	• What is your ethnic group?/ What is the ethnic group of the baby's father?
		  ○ Caucasian (white)
		  ○ Asian
		  ○ African
		  ○ Arabian
		  ○ Other including mixed race
			Parents were classified as Caucasian or non-Caucasian (all other groups).

Allergic disease in parents	Baseline questionnaire	For each parent:
		• Do you or did you ever suffer from pollen-related rhinitis (hay fever)?
		  ○ Was it medically diagnosed?
		• Do you or did you ever suffer from eczema?
		  ○ Was it medically diagnosed?
			Parents were defined as having self-reported, doctor diagnosed allergic disease if they reported medically diagnosed hay fever, eczema or asthma.

Variable	Source of data	Question/questions asked	Dichotomous classification
Allergic disease in parents	Baseline questionnaire	• Do you or did you ever suffer from asthma? ○ Was it medically diagnosed?	

Any maternal smoking	Baseline questionnaire	• Do you smoke?
		  ○ Yes
		  ○ No, ex-smoker
		  ○ No, never smoked
			Current smokers and ex-smokers were grouped together to create a dichotomous variable for ever versus never smoked.

Smoking during pregnancy	Baseline questionnaire	• Do you smoke?
		  ○ Yes
		  ○ No, ex-smoker
		  ○ No, never smoked
		• Did you stop smoking or reduce the number of cigarettes you smoked when you were pregnant?
		  ○ Yes, stopped completely
		  ○ Yes, reduced the number of cigarettes smoked
		  ○ No, continued to smoke at the same level
		  ○ I had already stopped before becoming pregnant
			Mothers were classified as having smoked during pregnancy if they answered yes or no, ex-smoker to 'Do you smoke?' and reported that they had reduced the number of cigarettes smoked or continued smoking at the same level when they were pregnant.

Mother smoking at one-year follow-up	One-year data	• Do you smoke?
		  ○ No
		  ○ Yes, daily
		  ○ Yes, occasionally
			Daily and occasional smokers were grouped together to differentiate smokers from non-smokers.

Day care at any time in first two years of life	One and two-year data	• Does your child attend day care or a nursery?
		  ○ Yes
		  ○ No
			If parents replied yes to this question in the time frame for one-year data, two-year or both children were classified as having attended day care in the first two years of life.

Variable	Source of data	Question/questions asked	Dichotomous classification

Upper respiratory tract infections	One and two-year data	• How often has your child had an upper respiratory tract infection in the last 12 months?
		  ○ None/once
		  ○ Occasionally (once every 3 months)
		• Often (once a month or more)
			Frequent upper respiratory tract infections were defined as one every 3 months (quarterly) or more.

Lower respiratory tract infections	One and two-year data	• How often has your child had a lower respiratory tract infection in the last 12 months?
		  ○ None/once
		  ○ Occasionally (once every 3 months)
		• Once a month or more
			Frequent lower respiratory tract infections were defined as one every 3 months (quarterly) or more.

Eczema in the first two years of life	One and two-year data	• Has your child had a rash or eczema that has lasted for at least 7 days or more? (Do not count regular nappy rash)
		  ○ Yes
		  ○ No
			If parents replied yes to this question in the time frame for one-year data, two-year data or both, children were classified as having eczema in the first two years of life.

2.4.2.2 Continuous Feeding Variables

The 12-and 24-month questionnaires included an extensive list of foods commonly found in the diets of children. Parents were asked if their child had tried each of these and if so, how old they were (in months) when they first tried each food. Parents were also asked if their child had ever been breastfed and if so, how old their child was (in months, weeks or days) when they stopped breastfeeding. Using this data, the age of each child when solids were first introduced and the overlap (in months) between breastfeeding and solids was determined. For overlap of breastfeeding/solids, values less than one were converted to zero.

2.4.2.3 Food Allergy

Children were defined as allergic or tolerant according to double-blind, placebo-controlled food challenge outcomes (Table 2).

Table 2 Determination of Double-Blind, Placebo-Controlled Food Challenge Outcomes

Active challenge day	**Placebo challenge day**	**Classification**
Positive	Negative	Allergic
Negative	Negative	Tolerant
Positive	Positive	Inconclusive *
Negative	Positive	Tolerant

*Inconclusive challenges were repeated.

Food allergy was diagnosed without a DBPCFC in children with a clear history of anaphylaxis and elevated allergen-specific serum IgE.

Children with food allergy were sub-divided into those with IgE-mediated and non-IgE mediated food allergy. IgE-mediated food allergy was defined as food allergy with evidence of allergic sensitisation i.e. a positive skin prick test (≥3mm wheal) or positive specific IgE (≥0.35 kU/l) to the triggering food at any time during follow up. Non-IgE mediated food allergy was defined as food allergy without any evidence of allergic sensitisation.

2.4.3 Outcome Variables

Wheeze in the second year of life was the primary outcome for this analysis. Questions relating to wheeze included: 'In the last 12 months, has your child had wheezing or whistling in the chest?' (12-month questionnaire) and 'Between the ages of 13 and 24 months, has your child had

wheezing or whistling in the chest when they did not have a cold?' (24-month questionnaire). Children were defined as having wheeze in the second year of life if parents answered yes to either of the above questions within the specified time range for two-year data. A secondary analysis comparing those with recurrent wheeze (defined as wheeze in both the first and second years of life) to a never wheezed group was undertaken to validate the study findings.

Recurrent wheeze (wheeze in both the first and second years of life) was initially proposed as the primary outcome for this analysis. This was to avoid the inclusion of infants with wheeze secondary to a single respiratory tract infection. A variable was created to distinguish between infants who had not wheezed at any time during the first two years of life (never wheezed), those who had only wheezed in the first year of life, those who had only wheezed in the second year of life and those with recurrent wheeze. To allow Poisson regression analysis, a dichotomous variable for those who had never wheezed and those with recurrent wheeze was also created.

The prevalence of recurrent wheeze was, however, <1% in three centres (Vilnius, Lodz and Athens), making it difficult to evaluate risk factors for wheeze in these centres. Therefore, wheeze in the second year of life was used as the primary outcome. The 24-month questionnaire (from which most two-year data was derived) specifically referred to wheeze without colds. Therefore, this approach also focused on children with multi-trigger wheeze (who are more likely to develop asthma)[18] rather than infants with wheeze secondary to a single respiratory tract infection.

2.4.4 Describing the Cohort

The baseline characteristics and exposures of participants were described for the whole cohort, separately for each centre and separately for those with and without wheeze in the second year of life. Percentages for each variable were calculated using the number of infants for whom data were available. Differences between centres and wheeze groups were tested using the Chi-squared test (for dichotomous/categorical variables), the one-way ANOVA or T-Test (for continuous, normally distributed variables) and the Kruskal-Wallis or Mann-Whitney U test (for continuous, non-normally distributed variables). The baseline characteristics of those with follow up data at 2 years were compared to the baseline characteristics of the whole cohort to identify potential follow-up bias.

2.4.5 Poisson Regression

Poisson regression was used to identify risk factors for wheeze in the second year of life. This form of regression was chosen because it allows relative risk ratios (rather than odds ratios, which may overestimate risk when an outcome is common) to be determined.[141]

Negative binomial regression was trialled as an alternative to Poisson regression. However, when using negative binominal regression, the dispersion parameter was 0 suggesting that a Poisson model was equally suitable.

2.4.6 Multivariable Analysis

Initially all exposure variables were entered into a multivariable model. Due to missing data, this meant that only 3503 participants were included. Therefore, a model including only variables with a p-value less than 0.1 in univariate analysis and any potentially key risk factors for wheeze (food allergy, feeding during infancy and cigarette smoke exposure) was generated instead (primary model). To account for heterogeneity between centres (in terms of both baseline factors and potential risk factors for wheeze) a dummy variable for study centre variable was added to this model. The effect of this variable was dependent on the order in which the centres were labelled i.e. in random order or according to the prevalence of wheeze. It was decided to use Reykjavik (the centre with the highest prevalence of wheeze) as the reference centre (taking a value of 1) and label the other countries from 2 to 9 in descending order of wheeze prevalence.

Three alternative multivariable models were generated in a sensitivity analysis. Sensitivity model one was generated by applying backward deletion to the primary model i.e. variables were sequentially removed from the primary model (starting with the variable with the weakest association with wheeze in the second year of life) until only those with a p-value less than 0.05 remained in the model. Backward deletion can be performed in STATA using the 'stepwise' command. However, this restricts the model to cases included in the full model. Therefore, backward deletion was performed manually. Sensitivity model two included all variables with a p-value less than 0.1 in univariate analysis and any potentially key risk factors for wheeze. Sensitivity model three was generated by applying backward deletion (as described above) to sensitivity model two. Neither sensitivity model two nor sensitivity model three included the 'study centre' variable. Finally, significant associations from the primary model were entered into a separate multivariable model to examine their importance in individual centres.

Chapter 3: UBIOPRED Methods

3.1 Study Design

The UBIOPRED project was a private-public partnership, within the framework of the Innovative Medicines Initiative (IMI), which was set up in 2009 to address knowledge gaps in severe asthma. As part of the UBIOPRED project, a multi-centre, prospective observational cohort study following the life course of asthma was undertaken. This aimed to use clinical features, physiological measurements, traditional biomarkers and omics technologies (transcriptomic, proteomic, lipidomic and metabolomic) to identify phenotypes of severe asthma in both adults and children.[142,143] The methods used and baseline characteristics of the participants have previously been described in the following papers:

Shaw D, Sousa AR, Fowler S, Fleming L, Roberts G, Corfield J et al. Clinical and inflammatory characteristics of the European UBIOPRED adult severe asthma cohort. *Eur Respir J* 2015; 46: 1308-1321.[143]

Fleming L, Murray C, Bansal AT, Hashimoto S, Bisgaard H, Bush A et al. The burden of severe asthma in childhood and adolescence: results from the paediatric UBIOPRED cohorts. *Eur Respir J* 2015; 46: 1322-33.[142] (I was one of 24 authors for this paper)

Children with asthma or preschool wheeze were recruited from seven centres in five European counties. These included:

- The University of Southampton, Southampton, United Kingdom
- Imperial College London, London, United Kingdom
- The University of Manchester, Manchester, United Kingdom
- The Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands
- The Centre for Allergy Research, Karolinska Institutet, Stockholm, Sweden
- University Hospital Inselspital, Bern, Switzerland
- Copenhagen University Hospital, Copenhagen, Denmark

Adults were also recruited from the first six of these plus ten other centres including:

- University of Catania, Catania, Italy
- Department of Medicine Jagiellonian University Medical College, Krakow, Poland
- Semmelveis University Department of Pulmonology, Budapest, Hungary
- Universite de la Mediterranee, Marseille, France

- Nottingham University Hospitals, Centre for Respiratory Research, Nottingham, United Kingdom
- Haukeland University Hospital, Bergen, Norway
- Fraunhofer Institute of Toxicology and Experimental Medicine, Hannover, Germany
- Department of Respiratory Medicine and Allergy, University Hospital, Umea, Sweden
- Hvidore Hospital, Hvidore, Denmark
- Department of Pharmacology, Faculty of Medicine Universita Cattolica del Sarco Curore, Rome, Italy

As outlined in Figure 3, all partcipants underwent a screening assessment (to assess eligibility for the study) followed by a baseline assessment within 28 days. Children with severe asthma or wheeze were reviewed at 12-18 months after enrolment in the study (longitudinal visit 2). 6-12 months after longituidinal visit 2, participants or their parents were also contacted by telephone to obtain information on asthma control.

The majority of adults with severe asthma were also reviewed once at 12-18 months after enrolment (longitudinal visit 2) and were contacted 3-6 months later by telephone or post to obtain information on asthma control. A small number of adults with severe asthma (who followed an earlier version on the study protocol) underwent two longitudinal visits. Longitudinal visit 1 occurred 3-6 months after enrolment and longitudinal visit 2 occurred 12-18 months after enrolment.

Children and adults were also invited to attend their local study centre if they experienced an exacerbation.

The study was approved by each centre's local ethics committee (Appendix B.1 and Appenidx B.2) and written, informed consent was obtained from all participants or their parents. Where appropriate, children also gave assent.[142,143]

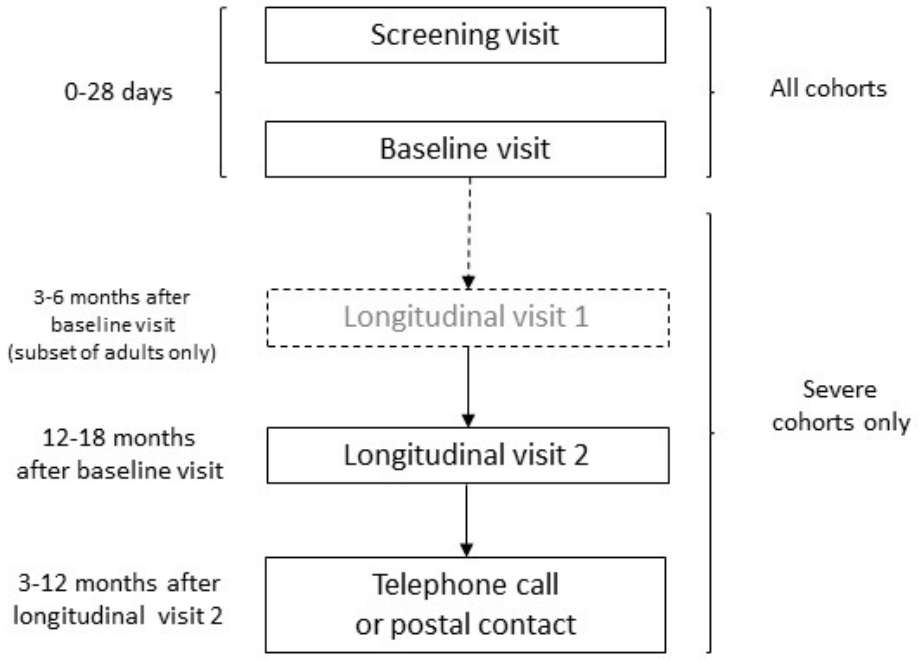

Figure 3 Visit Schedule for UBIOPRED Participants

3.2 Study Participants

3.2.1 Paediatric Particpants

Four paediatric cohorts were established by approaching the parents of children with preschool wheeze and asthma attending routine clinic appointments:

- Cohort A- School aged children (aged 6 to 17 years) with severe asthma (SA).
- Cohort B- School aged children (aged 6 to 17 years) with mild to moderate asthma (MMA).
- Cohort C- Preschool children (aged 1 to 5 years) with severe wheeze (SW).
- Cohort D- Preschool children (aged 1 to 5 years) with mild to moderate wheeze (MMW).

A full description of the cohorts is outlined in Table 3.

Table 3 Inclusion Criteria for the UBIOPRED Paediatric Cohorts

Cohort	Therapy	Disease Control	Diagnostic Criteria	Other
Cohort A: - School age - Severe asthma	- High dose ICS (≥500mcg FP or ≥ 800mcg BUD daily or equivalent) PLUS - A trial of at least two other controller medications e.g. leukotriene receptor antagonist or long-acting beta-agonist OR - Daily/alternate daily OCS OR - Treatment with omalizumab	- Persistent symptoms (at least 50% of days) and need for reliever treatment ≥ 3 times per week AND/OR - Frequent, severe exacerbations (≥2 in the past year or ≥3 in the past 2 years requiring hospital attendance or high dose OCS or ≥1 in the past year requiring PICU admission) AND/OR - Z-score FEV_1 < -1.96 (post bronchodilator or post steroid trial) AND/OR	- Airway hyperresponsiveness (PC_{20} <8mg/ml) OR - Bronchodilator reversibility (improvement in FEV1 >12% or 200ml after inhalation of ≥ 400mcg salbutamol) OR - Spontaneous variability in FEV1 ≥ 12% or 200ml over the past year OR - Diurnal variability in PEF≥ 15% in a properly conducted trial and with a compatible clinical picture	- Under the care of a respiratory paediatrician for ≥ 6 months

Cohort	Therapy	Disease Control	Diagnostic Criteria	Other
Cohort B: - School age - Mild to moderate asthma	- Low to moderate dose ICS (≤250mcg FP or ≤ 400mcg BUD daily or equivalent) PLUS - No more than one other controller medication	- Controlled asthma characterised by all of the following in the last 4 weeks: o Daytime symptoms ≤ 2 times per week o No limitation of activities o No nocturnal symptoms o Use of reliever medication ≤ 2 times per week o FEV_1 ≥ 80% predicted OR - Partically controlled asthma characteried by one or two of the following in the last 4 weeks: o Daytime symptoms ≥ 2 times per week o Any limitation of activities o Any nocturnal symptoms o Use of reliever medication ≥ 2 times per week o FEV_1 < 80% predicted	As for cohort A.	
Cohort C: - Preschool age - Severe wheeze	- High dose ICS (≥200mcg FP or ≥ 400mcg BUD daily or equivalent) and a leukotriene receptor antagonist OR - A failed trial of the above OR - Daily or alternate daily OCS	- Persistent symptoms (at least 50% of days) and need for reliever treatment ≥ 3 times per week AND/OR - Frequent, severe exacerbations (≥2 in the past year or ≥3 in the past 2 years requiring hospital attendance or high dose OCS or ≥1 in the past year requiring PICU admission)	- A history of breathlessness or wheeze.	- Under the care of a respiratory paediatrician for ≥ 6 months

Cohort	Therapy	Disease Control	Diagnostic Criteria	Other
Cohort D: - Preschool age - Mild to moderate wheeze	- No treatment or low dose ICS (≤100mcg FP or ≤ 200mcg BUD daily or equivalent) AND/OR - A leukotrience receptor antagonist	- Controlled disease characterised by all of the following in the last 4 weeks: ○ Daytime symptoms ≤ 2 times per week ○ No limitation of activities ○ No nocturnal symptoms ○ Use of reliever medication ≤ 2 times per week OR - Partially controlled disease characteried by one or two of the following in the last 4 weeks: ○ Daytime symptoms ≥ 2 times per week ○ Any limitation of activities ○ Any nocturnal symptoms ○ Use of reliever medication ≥ 2 times per week	- A history of breathlessness or wheeze.	

3.2.1.1 General Inclusion/Exclusion critera:

The following inclusion criteria applied to all cohorts:

1. Written informed consent from a person with parental responsbility prior to enrolment plus assent of the child where appropriate.
2. Aged 1-17 years at screening.
3. Parents/guardian and where appropriate, participant able to read and understand the study materials.

Exclusion criteria were as follows:

1. Deemed unfit for study participation following a screening assessment by a trained physician.
2. A history of drug or other allergy , which in the opinion of the responsible physician contraindicates participation.
3. Pregancy , lactation, up to 6 weeks post partum or up to 6 weeks post cessation of breastfeeding.
4. Risk of non-compliance with study procedures.
5. Prematurity (gestation ≤37 weeks).
6. Recent (within 3 months) participation in a study investigating new drugs or involving invasive procedures.

3.2.2 Adult Participants

Patients with severe and mild to moderate asthma were identified from existing patient cohorts at each study centre and were approached at the time of routine clinic visits. Healthy controls were recruited using advertisments in the local press. Once again, four cohorts were established:

- Cohort A- Non-smoking adults with severe asthma (SAn)
- Cohort B- Smokers and ex-smokers with severe asthma (SAs/ex)
- Cohort C- Non-smoking adults with mild to moderate asthma (MMAn)
- Cohort D- Non-smoking, healthy controls (HC)

These are described in Table 4. For cohorts A, B and C asthma was defined as a history of wheeze occurring spontaneously or on exertion and airway hyperresponsiveness (PC_{20} <8mg/ml), bronchodilator reversibility (improvement in FEV1 >12% or 200ml after inhalation of ≥ 400mcg salbutamol), diurnal variability in PEF amplitude > 8% of mean or a decrease in FEV1 >12% predicted or 200ml within 4 weeks after tapering maintenance treatment.

Table 4 Inclusion Criteria for the UBIOPRED Adult Cohorts

Cohort	Therapy	Disease Control	Smoking	Other
Cohort A: - Non-smokers - Severe asthma	- High dose ICS (≥1000 mcg FP daily or equivalent) ± OCS PLUS - One other controller medication	- Uncontrolled symptoms according to the GINA guidelines i.e. three or more of the following in any of preceding 4 weeks: • Daytime symptoms ≥ 2 times per week. • Any limitation of activities. • Nocturnal symptoms ≥ once a week. • Need for reliever treatment ≥ 2 times per week. • Pre-bronchodilator FEV_1 < 80% predicted or personal best.	- Non-smoker for at least 12 months with a smoking history of ≤ 5 pack years. *	- Under the care of a respiratory specialist for ≥ 6 months.
Cohort B: - Smokers or ex-smokers - Severe asthma	- As for cohort A.	- As for cohort A.	- Current smoker OR - Ex-smoker with a smoking history of ≥ 5 pack years.*	- As for cohort A.

Cohort	Therapy	Disease Control	Smoking	Other
Cohort C: - Non-smokers - Mild to moderate asthma	- Low dose ICS (< 500mcg FP daily or equivalent)	- Controlled disease characterised by all of the following in the last 4 weeks: o Daytime symptoms ≤ 2 times per week o No limitation of activities o No nocturnal symptoms o Use of reliever medication ≤ 2 times per week o Pre-bronchodilator FEV_1 ≥ 80% predicted OR - Partially controlled disease characterised by one or two of the following in the last 4 weeks: o Daytime symptoms ≥ 2 times per week o Any limitation of activities o Any nocturnal symptoms o Use of reliever medication ≥2 times per week o Pre- bronchodilator FEV_1 < 80% predicted	- Non-smoker for at least 12 months with a smoking history of ≤ 5 pack years.*	
Cohort D: - Healthy controls - Non-smokers	- None	- No history of asthma or wheeze. - Pre-bronchodilator FEV_1 ≥ 80%.	- Non-smoker for at least 12 months with a pack year history of ≤ 5 years. *	- No other chronic respiratory disease.

*Pack years= Number of cigarettes smoked per day/20 x number of years smoked.

3.2.2.1 General Inclusion/Exclusion Criteria:

Participants were eligible for inclusion in the study if they met the following criteria:

1. Able to give informed, written consent to participate in the study.
2. Aged 18 years or older at the screening visit.
3. Able to complete the study and all measurements.
4. Able to read and understand all study related materials.
5. If enrolled in other studies, participants were only allowed to enrol or continue to participate if permission from the Scientific Board was obtained.

Exclusion criteria were as follows:

1. Deemed unfit for study participation by a trained physician either because of the risk to the subject or the influence the subject may have on the study results.
2. History of recreational drug use or allergy, which in the opinion of a trained physician contraindicates participation.
3. Pregnancy, lactation, up to 6 weeks post-partum or up to 6 weeks post cessation of breastfeeding.
4. Recent participation (within 3 months) in a study investigating a new molecular entity/drug or in a study involving invasive procedures.
5. Risk of non-compliance with study procedures.
6. Recent history of incapacitating psychiatric illness.

3.3 Study Visits

At the screening visit, the inclusion and exclusion criteria were used to assess eligibility for the study. Data on demographics, current and past medical history (including a detailed asthma history), medications, asthma control and family history were collected. All participants enrolled in the study subsequently underwent a baseline visit at which a series of assessments were undertaken. In children and adults, these included spirometry, bronchodilator reversibility testing, exhaled nitric oxide (eNO) measurement, plethysmography, forced oscillation technique (FOT), induction of sputum (where possible), skin prick testing, collection of blood samples for specific IgE, genetics and biomarkers and collection of urine samples for biomarkers and cotinine levels. In addition, adults were invited to attend for a bronchoscopy and a high-resolution computed tomography (CT) scan. In all participants, validated questionnaires were used to assess asthma control and quality of life.

In order to capture exacerbations, participants with severe disease (paediatric cohorts A and C and adult cohorts A and B) were asked to contact their local study centre in the following situations:

- If they experienced an increase in symptoms (wheeze or breathlessness), an increase in short-acting bronchodilator use or a fall in morning peak expiratory flow (PEF) of >20% of baseline (personal best) for 2 or more days.
- If they had been assessed by a non-study physician, resulting in a temporary change in treatment particularly initiation of systemic corticosteroids or an increase in maintenance dose of systemic corticosteroids.
- If they had attended the emergency department or been hospitalised due to asthma.

Participants experiencing an exacerbation were subsequently invited to attend their local study centre for a physical examination, spirometry and bronchodilator reversibility testing. Asthma control and quality of life were also assessed using age appropriate questionnaires. All exacerbations were managed according to routine clinical practice.

At each longitudinal visit, participants or their parents were asked how many exacerbations they had experienced since their last visit or how many exacerbations they had experienced in the last 12 months (in the case of adults who only underwent one longitudinal visit). Details of asthma exacerbations (not previously captured) and current asthma medications were collected. Specifically, the treatment location and duration of each exacerbation was recorded, along with the treatment received. At longitudinal visit 2, some of the assessments undertaken at baseline were also repeated including spirometry, bronchodilator reversibility testing and eNO measurement

3.4 Clinical Assessments

To ensure consistency between study centres, all assessments were performed according to agreed standard operating procedures (SOPs), training in which was provided before the study commenced. Data was collected through an electronic case report form (eCRF). Only the clinical assessments relevant to this analysis are described below.

3.4.1 Study Questionnaires

3.4.1.1 Demographic Details

Demographic data collected included age, gender, ethnicity, education, occupation (adults only) and residential location.

3.4.1.2 Asthma/Respiratory History

Details of participants' asthma history were obtained including the age of onset, ICU admissions, medication use in the past 12 months, the number of exacerbations experienced in the past year and for each exacerbation, the treatment location and treatment received. Symptom triggers such as respiratory tract infections, animals, exercise and pollutants were also explored.

3.4.1.3 Other Medical Problems

Participants or their parents were asked if they had ever been diagnosed with a range of medical conditions including hay fever, eczema, allergic rhinitis, cardiac disease, diabetes, vocal cord dysfunction, gastro-oesophagel reflux disease (GORD) and nasal polyps. If participants or their parents reported any of these conditions, the age of onset (<2 years, 2 to ≤ 17 years, 18 or more years) and whether the condition was currenly active was recorded.

3.4.1.4 Food Allergies

Participants or their parents were asked if they had ever experienced symptoms suggestive of IgE-mediated food allergy i.e. urticaria, angioedema, pruritis, throat tightness, stridor, chest tightness or wheeze within two hours of contact with food. If so, they were asked which food/foods triggered the symptoms, if they had been diagnosed with an allergy to this food and at what age the symptoms occurred. This information along with the results of skin prick testing and/or specific IgE was used to determine the presence of food allergies.

3.4.1.5 Smoke Exposure

Details of participants' smoking habits and second-hand smoke exposure were obtained.

3.4.2 Physical Examination

At the baseline and longitudinal visits, a physical examination including measurement of height, weight, heart rate (HR), respiratory rate (RR) and blood pressure (BP), and auscultation of the chest was undertaken. Height and weight measurements were used to determine participants' body mass index (BMI). Paediatric height, weight and BMI values were converted to z-scores for statistical analysis.

3.4.3 Asthma Control

This was assessed using the Asthma Control Questionnaire (ACQ), Asthma Control Test (ACT) or Children's Asthma Control Test (C-ACT) depending on participants' age.

The ACQ was the first validated measure of asthma control to be developed.[121] It includes five questions about asthma symptoms over the past week, one question about broncodilator use over the past week and FEV_1. The items are equally weighted (each with a score of 0 to 6). The ACQ score is reported as the mean of either the five symptom items (ACQ5 score) or all seven items (ACQ7 score). A mean score of 0 indicates well controlled asthma and a mean score of 6 indicates extremely poorly controlled asthma. The ACQ is only validated for use in adult patients.

The ACT is a validated measure of asthma control in children aged 12-18 years. It comprises five questions which assess asthma symptoms over a four-week period and produce a score ranging from 0 to 25. Higher scores indicates better disease control.[144] The C-ACT is a similar tool validated for use in younger children (aged 4-11 years). This comprises seven questions (three of which are completed by the child's parents and four which are completed by the parent and child together). C-ACT scores range from 0 to 27. Once again, higher scores indicate better disease control.[145] To allow joint analysis of ACT and C-ACT scores in the school-aged cohorts, data were transformed to create a combined score. Asthma control z-scores were then generated.

3.4.4 Quality of Life

The Paediatric Asthma Quality of life Questionnaire (PAQLQ) was used in school-age children (paediatric cohorts A and B) and the Paediatric Asthma Caregivers Quality of Life Questionnaire (PACQLQ) was used in preschool children (paediatric cohorts C and D).

The PAQLQ includes 23 questions in 3 domains (symptoms, activity limitation and emotional function). Children are asked to think about how they have felt during the previous week and to respond to each question using a 7-point scale (where 7 is not bothered at all and 1 is extremely bothered). The overall PAQLQ score is the mean of all 23 responses and the individual domain scores are the means of the items in those domains. The lower a child's PAQLQ score, the lower their quality of life.[146]

The PACQLQ was developed by the same group as the PAQLQ and was validated using the same methodology. It consists of of 13 questions; four concerning activity limitation and nine concerning emotional function. Parents are asked to recall the impact their child's asthma has had on them during the previous week and score each question using a 7-point scale (where 7 represents no impairment and 1 represents severe impairement). Once again, the overall score is

the mean of all responses.[147] The PACQLQ is only validated for use in children aged 7-17 years. However, there is no equivalent tool for assessing quality of life in younger children.

In adults, several questionnaires relating to quality of life were administered including the asthma quality of life questionnaires (AQLQ),[148] the Hospital Anxietry and Depression Scale (HADS) and the Sino-Nasal Outcomes Test (SNOT20). In this analysis, only AQLQ scores were considered. The AQLQ includes 32 questions in 4 domains (symptoms, activity limitation, emotional function and environmental exposures) with 2-week recall. As for the PAQLQ and PACQLQ, patients respond to each question on a 7-point scale (where 7 is no impairment and 1 is severe impairment) and the overall score is the mean of all responses.

3.4.5 Medication Adherence

The Medication Adherence Report Scale (MARS) was used to assess adherence in all cohorts. This is a 10-item questionnaire which assesses both intentional and accidental non-adherence. Questions are framed as negative statements e.g. 'I forget to take my medication' (to minimise social desirability bias) and medication use is rated on a 5-point scale with 1 indicating always and 5 indicating never. Therefore, a higher MARS score indicates greater medication adherence.[149]

3.4.6 Spirometry

This was performed according to ATS/ERS guidelines in all adults and school aged children.[150] Spirometry was also attempted in some preschool children. A portable spirometer calibrated with a three litre syringe was used. Short-acting bronchodilators were withheld for 4 hours and long-acting bronchodilators for 12 hours prior to testing. The best values for forced vital capacity (FVC) and forced expiratory volume in 1 second (FEV_1) were selected from a minimum of three acceptable manoeuvres. A maximum of eight attempts was allowed. Spirometry was repeated 10-15 minutes after administering 400mcg of salbutamol via a metered dose inhaler and volumatic spacer to allow FEV_1 bronchodilator reversibility to be determined.

3.4.7 Skin Prick Testing

Skin prick testing to a panel of six common allergens (mixed grass pollens, mixed tree pollens, dog, cat, aspergillus and a mixture of Dermatophagoides pteryonyssinus and Dermatophagoides farinae) was performed in all participants. In some centres, other aeroallergens such as cockroach and Parietaria were also tested. Skin prick testing to specific foods was performed if participants reported a history suggestive of food allergy. As per standard practice, positive (histamine 10mg/ml) and negative (normal saline) controls were used. Skin prick test solutions were placed

at least 2cm apart on the forearm or back at sites marked with the first letter of the allergen being tested. A single-headed lancet was then pushed through each drop at 90 degrees to the skin without drawing blood. The negative control was always tested first and the positive control last. Surplus fluid was removed from all sites simultaneously by placing a piece of tissue paper over the drops. Results were read (using a millimetre ruler or reaction gauge) 15 minutes after completing the positive control. A positive skin prick test was defined as a wheal ≥ 3mm in the presence of positive (≥3mm) and negative (0mm) controls.

3.4.8 Measurement of Total and Specific IgE

Total IgE and specific IgE to six common aeroallergens (as listed above) were measured (Thermo Fisher, Uppsala, Sweden). IgE to foods were also measured if participants reported a history suggestive of food allergy.

3.4.9 Immuno Solid-phase Allergen Chip (ISAC)

Levels of specific IgE to 112 allergen components from 51 sources were measured using the ImmunoCAP ISAC chip (Thermo Fisher Scientific, Uppsala, Sweden).[73] This is a biochip which requires only a few microliters of serum or plasma. Purified allergen components are immobilised on the biochip. In a two-step assay, antibodies from the patient bind to the immobilised allergen components. After a short washing step, allergen bound antibodies are detected by a fluorescence-labelled antibody. Test results are measured with a biochip scanner and are reported via Phadia MIA software in ISAC standardised units (ISU).

3.5 Outcomes

3.5.1 Allergic Sensitisation and Atopy

For each allergen tested, participants were considered to be sensitised if they had a specific IgE level ≥ 0.35 kU/l or a wheal diameter ≥3mm on skin prick testing.

Participants were classified as atopic if they were sensitised to one or more of the following aeroallergens: tree pollen, grass pollen, cat, dog, house dust mite and mould.

3.5.2 Food Allergy

Possible food allergy: Participants were defined as having possible food allergy if they had a history of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with a food plus a positive skin prick test (≥3mm wheal diameter) or positive specific IgE (≥ 0.35 kU/l) to that food.

Highly likely food allergy: A sensitivity analysis was performed using higher cut-off points. For this, food allergy was considered highly likely if participants had a history of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with a food plus a wheal diameter ≥ 5mm to that food on skin prick testing or a specific IgE level ≥10.0.

The food allergy status of participants with a history suggestive of food allergy but no data on sensitisation was treated as unknown.

3.5.3 Exacerbations

Exacerbations were defined as follows according to the ATS/ERS Taskforce 2009:[112]

Moderate exacerbation: A deterioration in symptoms, lung function and/or an increase in bronchodilator use for at least 2 days, but not severe enough to require systemic corticosteroids or hospitalisation.

Severe exacerbation: An exacerbation involving at least one of the following:

- Systemic corticosteroids or an increase from a stable maintenance dose for at least 3 days.
- Hospitalisation or emergency department visit requiring systemic corticosteroids (any duration).

Life threatening exacerbation: An intensive care unit (ICU) admission due to asthma.

For paediatric participants and adults who underwent two longitudinal visits, the exacerbation rate (the number of exacerbations per year) was calculated from the number of exacerbations between the baseline visit and longitudinal visit 2 divided by duration of follow up (months) multiplied by 12. This was calculated separately for moderate, severe (hospitalised and non-hospitalised) and life-threatening exacerbations to allow for sub-group analyses.

Adult participants who underwent one longitudinal visit were asked how many exacerbations they had experienced in the previous year.

3.5.4 Clinical Clusters

Cluster analyses were undertaken separately for paediatric and adult participants. The adult cluster analysis was undertaken by Lefaudeux et al. and is now published.[151] The paediatric cluster analysis was undertaken by Hashimoto S, Brinkman P et al. using the exacerbation statistics generated for this thesis; the manuscript is in preparation.

3.5.4.1 Adult Participants

For the adult participants, cluster analysis focused on 8 clinical variables accessible to the general practitioner including age of onset of asthma symptoms, pack years of cigarette smoking, body mass index (BMI), FEV_1 percent predicted, FEV_1/FVC ratio, ACQ5 score, number of exacerbations in the previous year and the daily dose of oral prednisolone or equivalent. 418 (out of 509) participants with a complete set of data for these 8 variables were split randomly into a training set (266 participants) and a validation set (152 participants). Partition around medoids (PAM) clustering was applied to both the training and validation sets. For the training set, this identified four stable clusters of asthmatic patients:

- T1: Patients with well-controlled moderate-severe asthma with normal FEV_1, low sputum eosinophilia, almost no oral corticosteroid (OCS) use and a high proportion of atopic participants.
- T2: Overweight to obese patients with late-onset severe asthma who smoked, had severe airflow obstruction and high levels of blood/sputum eosinophils.
- T3: Patients in this cluster were similar to those in cluster T2 but had no smoking history.
- T4: Obese female patients with severe uncontrolled asthma but normal lung function.

For the validation set, five stable clusters (V1, V2, V3, V4a and V4b) were identified. Cluster V1 was similar to cluster T1, cluster V2 was similar to cluster T2, cluster V3 was similar to cluster T3 and clusters V4a and V4b combined were similar to cluster T4. Full details of the characteristics of the training and validation set clusters can be found in the paper published by Lefaudeux et al.[151]

For the purposes of this analysis, the training and validation clusters have been combined to give four clusters:

- T1+V1= Cluster 1
- T2+V2= Cluster 2
- T3+V3= Cluster 3
- T4+T4= Cluster 4

3.5.4.2 Paediatric Participants

For paediatric participants, all variables that were available in at least 95% of participants were taken into account. A clustering strategy based on a combination of factor analysis of mixed data (FAMD) and partition around medoids (PAM) clustering was used. This generated 6 clusters with the following characteristics:

- Cluster 1: Pubertal female patients with atopy, high BMI, inhaled corticosteroid (ICS) use in all, poor lung function, frequent exacerbations and frequent oral corticosteroid (OCS) use.
- Cluster 2: Female preschool patients with less atopy, minimal ICS use, good quality of life and few exacerbations.
- Cluster 3: Pubertal male patients with atopy, low quality of life and high ICS use.
- Cluster 4: Male preschool patients with less atopy, low quality of life, high ICS use and frequent exacerbations.
- Cluster 5: Pre-pubertal male patients with atopy, good quality of life and high ICS use.
- Cluster 6: Male preschool patients with atopy, low quality of life, ICS use in all, frequent exacerbations and previous ICU admissions but good lung function.

3.5.5 ISAC Component Atopy Clusters

Hierarchical clustering was applied by Fontanella S, Howard R, Roberts G et al. to adults, school aged children and preschool age children with ISAC chip data. Data were available for 491/509 (96%) adults with asthma and 239/272 (88%) children with asthma/preschool wheeze. Four allergic sensitisation clusters were identified in school aged children and adults: A multiple sensitisation cluster, a house dust mite sensitisation cluster, a grass pollen sensitisation cluster and a miscellaneous sensitisation cluster. Only two clusters were identified in preschool children: A multiple sensitisation cluster and a house dust mite sensitisation cluster. Participants in the multiple sensitisation cluster were sensitised multiple components from different sources whilst participants in the miscellaneous sensitisation cluster had positive responses to a small number of components from a wide range of sources.

3.6 Statistical Analysis

Data from participants' electronic case report forms were uploaded onto the tranSMART system; a platform for sharing research data which is supported by the European Translational Information and Knowledge Management Services (eTRIKS) project.[143] Data from tranSMART were subsequently downloaded and transferred to STATA SE 14 (StataCorp, College Station, USA) for analysis.

3.6.1 Allergic Sensitisation and Allergic Disease

In the primary analysis, the prevalence of allergic disease and allergic sensitisation across the life course was studied in participants with mild to moderate and severe disease. Specifically, the prevalence of the following was compared in preschool children, school aged children and adults with mild to moderate and severe disease:

- Allergic conditions- Eczema, allergic rhinitis and hay fever.
- Food allergy- possible and highly likely (see section 3.5.2 for definitions).
- Sensitisation to tree pollen, grass pollen, dog, cat, house dust mite and mould.
- Atopy

The Chi-squared test was used for categorical variables, the one-way ANOVA test for continuous, normally distributed variables and the Kruskal-Wallis test for continuous, non-normally distributed variables. For food allergy and allergic sensitisation, pairwise comparisons were performed where differences were statistically significant ($p \leq 0.05$). Box plots were used to demonstrate differences in the number of allergens to which participants in each cohort were sensitised.

In a secondary analysis, participants were split according to age i.e. preschool children, school aged children and adults and comparisons were made between those with mild to moderate and severe disease.

3.6.2 Exacerbations

For each UBIOPRED cohort, the median exacerbation rate and 25^{th} and 75^{th} quartiles were calculated for all exacerbations, moderate exacerbations, severe (hospitalised and non-hospitalised) exacerbations and life-threatening exacerbations. As exacerbation rates were not normally distributed, differences between cohorts were tested using the Kruskal-Wallis test. If relevant, post hoc pairwise testing using the Mann-Whitney U-test was undertaken to determine

which cohorts differed from each other. Rates of exacerbations for each clinical cluster and each ISAC component atopy cluster were calculated and analysed in the same way. The proportion of participants in each cohort and each cluster who experienced any exacerbations during follow up was also calculated. Differences between cohorts and clusters were tested using the Chi-squared test.

The association between specific clinical characteristics (including demographic factors, the number of asthma exacerbations in the previous year, symptom triggers, allergic sensitisation, lung function and asthma control) and the rate of future severe exacerbations was assessed separately for adults, preschool and school aged children using negative binomial regression. Variables with a p-value <0.1 were entered into multivariable backward deletion models. Variables were sequentially removed from each model (starting with the variable with the weakest association with severe asthma exacerbations) until only those with a p-value less than 0.05 remained in the model. For children, multivariable models were generated separately for preschool and school aged children. Factors which were significant in the two models were subsequently entered into a combined model for all paediatric participants. A categorical variable to distinguish between preschool and school aged participants was also included. Backward deletion was applied until only factors with a p-value less than 0.05 remained in the model.

Once models containing factors with a p-value less than 0.05 had been generated, clinical cluster and ISAC component atopy cluster variables were added to determine whether these improve the ability to predict asthma exacerbations. For each age group, the clinical cluster with the largest number of participants was used as the baseline cluster (against which others were compared). For adults, this was cluster 4 and for children this was cluster 3. For the ISAC atopy component clusters, participants in each cluster were compared with non-sensitised individuals.

Chapter 4: EuroPrevall Results

4.1 Participants

The EuroPrevall birth cohort consisted of 12,049 infants. The baseline characteristics of the cohort are outlined in Table 5. 6189 (51.4%) of infants were male. The mean birth weight of all participants was 3.4kg (SD 0.51). Mode of delivery varied considerably between centres with the proportion of infants born via Caesarean section ranging from 2.5% in Madrid to 44.2% in Athens. Overall, the prevalence of allergic disease was 26.3% in participants' mothers and 21.0% in participants' fathers. It was lowest in Lodz (Poland) and Vilnius (Lithuania) and highest in Southampton (UK) and Reykjavik (Iceland). With the exception of the UK, the majority of families lived in an urban environment (84%). Once again there was considerable variation between centres. Most mothers (76%) had completed junior college/vocational education or higher. Fewest mothers had done so in Lodz (Poland), Vilnius (Lithuania) and Athens (Greece).

After excluding participants followed up outside the specified age ranges for one and two-year data, follow up data were available in 8174 infants (67.8%) at one year and in 8805 infants (73.1%) at two years. Follow up varied between centres with availability of one-year data ranging from 2% (Milan) to 90% (Berlin) and availability of two-year data ranging from 60.8% (Milan) to 83.5% (Lodz) (Table 6). 12-month questionnaires were completed in more infants from Milan than this analysis suggests. However, the dates on which most of these infants' 12-month questionnaires were completed were not available. Therefore, it was not possible to determine their ages at the time of data collection.

The baseline characteristics of those with two-year data were very similar to those without two-year follow up data (Table 7). However, a higher proportion of parents of children with two-year follow up data had a University/College education (45.9% compared to 31.4% of parents of children without two-year follow up data).

Table 5 Baseline Characteristics of the EuroPrevall Cohort by Centre

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
BASIC DEMOGRAPHICS AND BIRTH DETAILS										
Male gender % *	51.4	51.2	51.2	52.7	51.7	51.7	51.2	50.7	50.1	52.6
Gestation, weeks median (range) #	39 (34-44)	40 (34-44)	40 (34-43)	40 (34-43)	39 (34-43)	39 (34-42)	39 (34-42)	39 (34-43)	39 (34-43)	40 (34-44)
Birth weight, kg mean (SD) $	3.40 (0.51)	3.76 (0.51)	3.46 (0.52)	3.48 (0.51)	3.41 (0.49)	3.28 (0.50)	3.52 (0.44)	3.25 (0.43)	3.27 (0.51)	3.20 (0.46)
Birth length, cm mean (SD) $	51.5 (3.02)	51.7 (2.21)	52.8 (3.09)	50.9 (2.34)	51.3 (2.54)	54.2 (3.14)	52.7 (2.23)	49.3 (2.08)	49.3 (2.41)	50.5 (2.70)
Apgar score at 5 mins median (range) #	10 (7-10)	9 (7-10)	10 (7-10)	10 (7-10)	10 (7-10)	9 (7-10)	10 (8-10)	10 (7-10)	9 (7-10)	10 (7-10)
Single birth % *	97.9	98.7	97.7	98.4	96.4	96.1	99.2	99.6	98.2	96.7
Caesarean section % *	24.0	12.8	30.8	11.0	31.1	37.5	15.6	2.5	30.8	44.2
Caucasian mother % *	93.3	99.2	95.9	72.2	93.4	99.9	99.9	84.5	89.9	99.2
Caucasian father % *	92.7	98.4	97.0	69.7	90.0	99.3	99.5	84.8	90.4	99.4
Maternal age, years mean (SD) $	30.7 (5.21)	30.1 (4.81)	31.8 (5.18)	29.9 (4.82)	31.4 (5.41)	28.8 (4.43)	28.2 (5.20)	31.4 (5.10)	33.6 (4.66)	30.9 (4.86)
Paternal age, years mean (SD) $	33.3 (6.10)	32.2 (5.50)	34.1 (5.67)	33.0 (6.07)	34.6 (6.61)	31.1 (5.40)	30.9 (6.25)	33.7 (5.65)	36.1 (5.75)	34.8 (5.47)
FAMILIAL ALLERGIC DISEASE										
Maternal self-reported, doctor-diagnosed allergic disease %										
-Any *	26.3	44.5	51.4	36.5	35.3	9.7	5.9	24.8	23.7	14.0
-Asthma *	9.4	17.2	22.8	13.3	10.8	3.2	1.5	6.2	8.7	5.2
-Allergic rhinitis *	15.3	15.9	26.3	24.1	24.6	7.2	3.5	16.2	14.9	9.5
-Eczema *	11.7	28.0	28.1	14.5	13.4	1.7	2.6	10.1	9.6	2.0

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
Paternal self-reported, doctor-diagnosed allergic disease %										
-Any *	21.0	32.1	41.0	30.8	29.3	8,7	2.8	19.4	21.4	11.7
-Asthma *	7.2	9.9	19.4	9.8	8.7	2.1	0.8	5.2	8.7	3.7
-Allergic rhinitis *	14.1	15.4	22.8	20.2	23.6	7.2	2.1	15.1	15.7	8.8
-Eczema *	6.2	16.1	15.7	9.3	7.1	1.1	0.3	2.5	5.4	0.5
LIVING ENVIRONMENT										
Rural housing % *	16.1	7.2	76.1	0.6	2.2	22.4	13.6	12.3	8.6	7.9
Mould in house % *	9.9	7.6	10.7	17.5	11.4	2.0	10.2	4.6	9.3	21.5
Pets %										
-Any *	35.5	28.0	50.2	54.5	33.1	46.3	43.8	23.5	24.4	19.5
-Cat *	15.1	11.3	28.8	26.8	15.0	15.1	22.0	5.4	10.5	3.7
-Dog *	16.0	9.3	18.1	14.1	9.0	34.8	21.7	13.5	10.8	10.2
MATERNAL EDUCATION										
Basic not completed % *	5.9	0.8	0.9	1.1	11.2	6.2	6.9	12.3	0.5	11.1
Basic completed % *	18.2	15.1	10.8	11.8	10.8	27.2	19.1	24.9	12.8	31.0
Junior College/ vocational % *	34.0	30.7	30.2	45.6	37.7	15.7	21.6	62.8	40.3	23.3
College/ university % *	42.0	53.5	58.1	41.4	40.2	50.9	52.5	0.0	46.4	34.6

Study centre (n) = total number of infants recruited.

Any allergic disease was defined as asthma, allergic rhinitis or eczema.

Rural housing was defined as living in a village or the countryside and urban living was defined as living in a town or city.

Basic education was defined as completing 10 years in school.

*p <0.05 using Chi-squared to test differences between centres.

# p<0.05 using Kruskal-Wallis to test differences between centres.

$ p<0.05 using one-way ANOVA to test differences between centres.

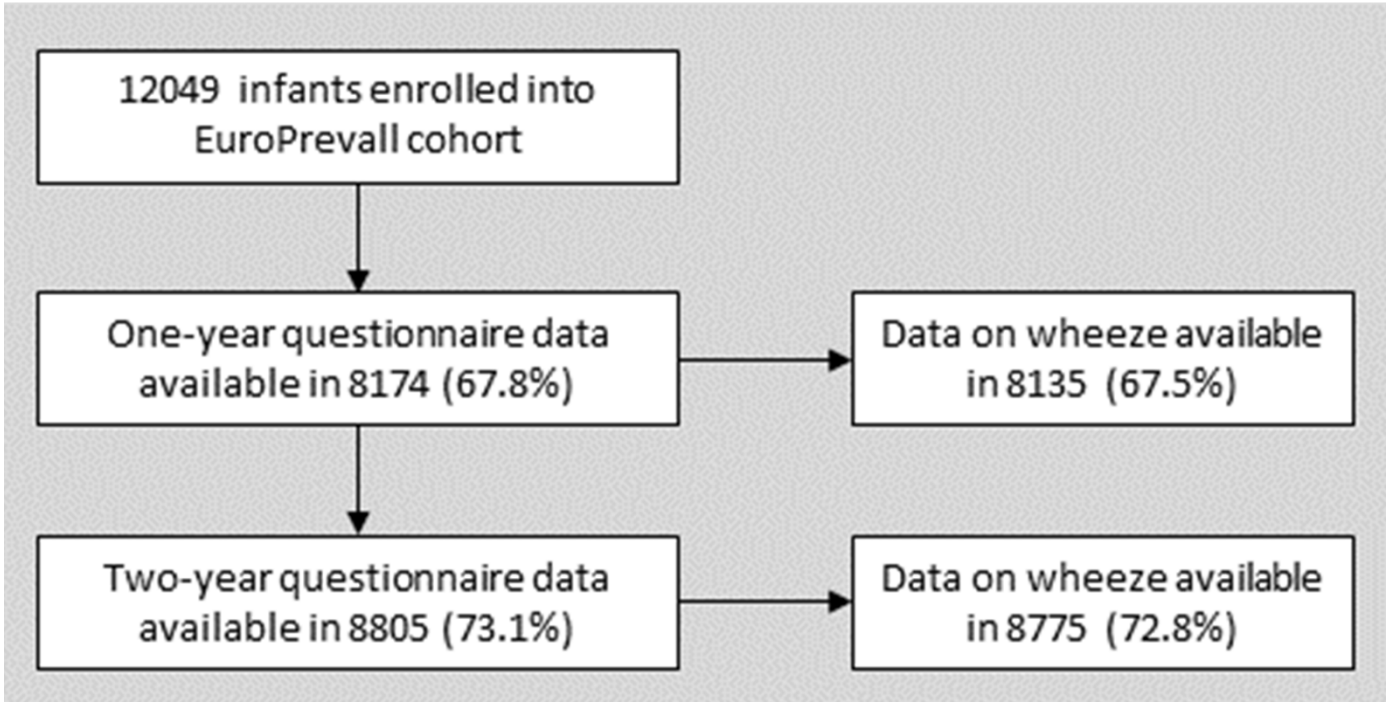

Figure 4 EuroPrevall Participants included in this Analysis

Table 6 Number of Participants Followed Up in each Centre

	All centres	Reykjavik	Southampton	Amsterdam	Berlin	Lodz	Vilnius	Madrid	Milan	Athens
Total number recruited	12049	1341	1140	976	1570	1513	1556	1387	1486	1080
Number with one-year follow up data n (%)	8174 (67.8)	1017 (75.8)	621 (54.5)	845 (86.6)	1406 (90.0)	1210 (80.0)	1168 (75.1)	1015 (73.2)	30 (2.0)	862 (79.8)
Number with two-year follow up data n (%)	8805 (73.1)	1078 (80.4)	766 (67.2)	624 (63.9)	1296 (82.6)	1263 (83.5)	1166 (75.0)	911 (65.7)	904 (60.8)	797 (73.8)
Number with one-year data, two-year data or both n (%)	9963 (82.7)	1245 (92.8)	882 (77.4)	887 (90.9)	1447 (92.2)	1339 (88.5)	1299 (83.5)	1387 (76.6)	912 (61.4)	890 (82.4)
Number with one-year and two-year data n (%)	7016 (58.2)	850 (63.4)	505 (44.3)	582 (59.6)	1255 (80.0)	1134 (75.0)	1035 (66.5)	864 (62.3)	22 (1.5)	769 (71.2)

One-year data= data collected between 6 and 18 months.

Two-year data= data collected between 18 and 30 months.

Table 7 Comparison of the Key Baseline Characteristics of Participants with and without Two-Year Follow Up Data

	Participants with two-year follow up data (n=8805)	Participants without two-year follow up data (n=3244)
BASIC DEMOGRAPHICS AND BIRTH DETAILS		
Male gender %	51.4	51.2
Gestation, weeks median, range	39 (34-44)	39 (34-44)
Birth weight, kg mean (SD)	3.42 (0.51)	3.37 (0.51)
Caesarean section %	24.6	22.4
Caucasian mother %	95.4	87.6
Caucasian father %	94.8	86.8
Maternal age, years mean (SD)	31.0 (5.00)	29.8 (5.64)
Paternal age, years mean (SD)	33.5 (5.89)	32.8 (6.59)
FAMILIAL ALLERGIC DISEASE		
Maternal self-reported, doctor diagnosed allergic disease %		
-Any	26.3	26.4
-Asthma	9.2	9.8
-Allergic rhinitis	15.2	15.6
-Eczema	11.9	11.3
Paternal self-reported, doctor diagnosed allergic disease %		
-Any	21.3	20.1
-**Asthma**	7.1	7.5
-Allergic rhinitis	14.4	13.5
-Eczema	6.2	6.2
LIVING ENVIRONMENT		
Rural housing %	16.2	15.8
Mould in house %	9.8	10.2
Pets %		
-Any	36.1	34.1
-Cat	15.5	14.0
-Dog	16.1	15.8
MATERNAL EDUCATION		
Basic not completed %	4.4	10.0
Basic completed %	16.6	22.5
Junior College/ vocational %	33.2	36.1
University/ college %	45.9	31.4

Any allergic disease was defined as asthma, allergic rhinitis and/or eczema.

Rural housing was defined as living in a village or the countryside and urban living was defined as living in a town or city.

Basic education was defined as completing 10 years in school.

4.2 Prevalence of Wheeze

The prevalence of wheeze in the second year of life across all centres was 7.8%, ranging from <3% in Lodz (Poland), Vilnius (Lithuania) and Athens (Greece) to 12% in Berlin (Germany), 13% in Southampton (UK) and 17% in Reykjavik (Iceland) (Table 8 and Figure 5). Large differences in the prevalence of wheeze in the first year of life and recurrent wheeze (wheeze in the first and second years of life) were also seen. The prevalence of recurrent wheeze was lowest in Lodz (1.7%), Vilnius (1.9%) and Athens (2.8%) and highest in Amsterdam (6%), Southampton (8%) and Reykjavik (10%).

4.3 Distribution of Potentially Key Risk Factors for Wheeze

Amongst children included in this analysis, the prevalence of food allergy ranged from 0.1% in Athens (Greece) to 3% in Southampton (UK). The majority of cases of food allergy were IgE-mediated (Table 9).

91% of all infants received some breast milk. The highest rates of breastfeeding were seen in Reykjavik (98%) and the lowest in the Amsterdam (81%). Wide variations in the mean duration of breastfeeding were seen, ranging from 4.3 months in Athens to 8.5 months in Reykjavik. The age at introduction of first solids appeared similar across centres (range 5.0-5.7 months) but differences between centres were statistically significant. In most centres, there was no overlap between breastfeeding and solids. However, in Milan, Berlin and Reykjavik, there was a median overlap of 1, 2 and 3 months respectively (Table 9).

Rates of maternal smoking (during pregnancy and at one-year follow up) varied considerably between centres (Table 9). Exposure to cigarette smoke during pregnancy was highest in Athens (18%) compared to <11% in all other centres. Maternal smoking at one-year follow up was also highest in Athens (33%), followed by Madrid (23%). This compares to 16% overall and <10% in Vilnius and Southampton. Smoking by other household smokers was 22% overall (range 43% to 3%).

Other factors examined included day care attendance, respiratory tract infections and eczema. Day care attendance in both the first and second years of life was much lower (<30%) in Lodz, Vilnius and Athens than the other centres. The rates of upper respiratory tract infections in the first two years of life were lowest in Vilnius (Lithuania), Madrid (Spain) and Athens (Greece). They were highest in Reykjavik (Iceland), Amsterdam (Netherlands) and Berlin (Germany), where over 80% of parents reported that their child had experienced three or more upper respiratory tract infections in each of the first two years of life. For lower respiratory tract infections, the lowest

rates were reported in Vilnius (Lithuania), Amsterdam (Netherlands) and Lodz (Poland). Although only 0.8% of participants in Athens (Greece) experienced three or more lower respiratory tract infections in the first year of life, this figure increased to 23.3% in the second year of life. In Reykjavik (Iceland), the prevalence of frequent respiratory tract infections was >20% in both the first and second years of life. Eczema was most common in Reykjavik (Iceland), Southampton (UK) and Amsterdam (Netherlands) affecting over 45% of infants. It was least common in Vilnius (Lithuania) and Athens (Greece).

Table 8 Prevalence of Wheeze in the First Two Years of Life by Centre

	All centres	**Reykjavik (n=1341)**	**Southampton (n=1140)**	**Amsterdam (n=976)**	**Berlin (n=1570)**	**Lodz (n=1513)**	**Vilnius (n=1556)**	**Madrid (n=1387)**	**Milan (n=1486)**	**Athens (n=1080)**
Wheeze in the first year of life % (95% CI)	13.5 (12.7-14.2)	33.9 (31.0-36.9)	31.6 (27.9-35.2)	26.1 (23.2-29.1)	9.1 (7.6-10.6)	11.4 (9.6-13.2)	0.5 (0.1-0.9)	4.1 (2.8-5.3)	**	2.0 (1.0-2.9)
Wheeze in the second year of life % (95% CI)	7.8 (7.2-8.3)	17.2 (15.0-19.5)	13.1 (10.7-15.5)	10.8 (8.3-13.2)	11.8 (10.0-13.5)	1.7 (1.0-2.4)	1.9 (1.1-2.7)	3.0 (1.9-4.1)	9.5 (7.6-11.4)	2.8 (1.6-3.9)
Recurrent wheeze (wheeze in the first and second years of life) % (95% CI)	3.1 (2.7-3.5)	10.0 (7.9-12.0)	7.9 (5.6-10.3)	6.4 (4.4-8.4)	1.8 (1.1-2.6)	0.8 (0.2-1.3)	0.3 (0.0-0.6)	2.0 (1.0-2.9)	**	0.4 (0.0-0.8)

Study centre (n) = total number of infants recruited.

$p<0.05$ for all variables using Chi-squared to test differences between centres.

**For Milan, the prevalences of wheeze in the first year of life and recurrent wheeze are not specified because one-year data was not available for most participants.

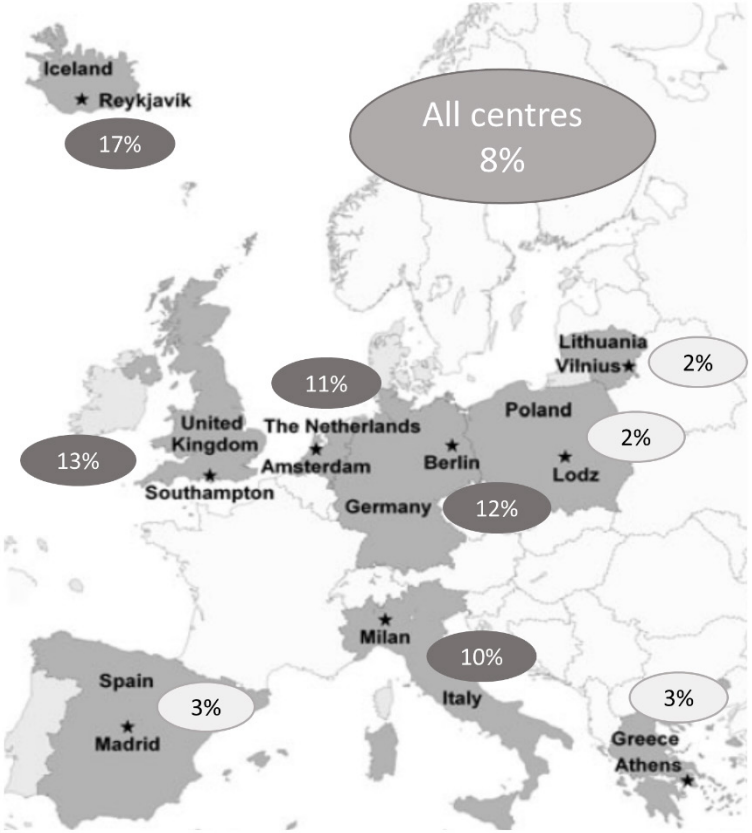

Figure 5 Map showing Study Centres and the Prevalence of Wheeze in the Second Year of Life in each Centre (adapted from Keil et al. *Allergy* 2010; 65) [135 (p.484)]

Table 9 Potential Risk Factors for Wheeze by Centre

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
FOOD ALLERGY										
Any food allergy diagnosed in first two years of life % *	1.3	2.1	3.0	2.3	1.0	1.2	0.5	1.5	0.8	0.1
IgE mediated food allergy % *	1.1	1.9	1.9	1.2	1.0	1.1	0.4	1.5	0.8	0.1
FEEDING										
Ever breastfed % *	90.8	98.4	89.8	81.3	95.8	90.5	94.9	86.1	91.0	83.1
Duration of breastfeeding, months mean (SD) $	6.1 (3.90)	8.5 (3.46)	5.4 (4.14)	4.6 (3.41)	6.4 (3.60)	6.1 (4.44)	5.7 (3.94)	5.3 (2.98)	7.1 (3.76)	4.3 (3.28)
Age at introduction of first solids, months mean (SD) $	5.3 (1.61)	5.3 (1.20)	5.1 (1.36)	5.2 (1.47)	5.5 (1.57)	5.0 (1.27)	5.7 (2.54)	5.1 (1.39)	5.6 (1.48)	5.3 (1.44)
Overlap of breastfeeding/solids, months median (range) #	0 (0-25.8)	3.2(0-18.4)	0 (0-23.5)	0 (0-15.4)	1.1(0-13.3)	0 (0-15.3)	0.1 (0-11.4)	0 (0-11.3)	2.1(0-11.6)	0 (0-25.8)
SMOKE EXPOSURE										
Mother ever smoked %*	41.7	38.4	41.8	42.9	52.7	34.0	40.6	39.0	40.0	46.4
Smoking at any time during pregnancy % *	9.6	7.7	6.7	10.5	10.3	8.8	7.5	10.9	7.8	18.1
Mother smoking at one year follow up % *	15.9	10.3	6.3	15.2	16.9	14.4	8.0	23.0	**	33.3
Other smokers in household % *	22.9	3.0	17.2	20.2	10.1	31.7	42.9	17.8	27.7	33.4

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
DAY CARE ATTENDANCE										
Day care in first year of life % *	32.2	61.3	50.0	72.7	38.2	6.5	2.0	40.0	**	3.3
Day care in second year of life % *	55.3	93.7	58.6	83.4	81.2	18.6	27.2	63.6	62.5	17.6
Day care at any time in first two years of life % *	63.7	97.0	73.7	90.7	82.9	22.8	30.6	68.0	**	18.9
RESPIRATORY TRACT INFECTIONS										
Frequent URTIs (≥ quarterly) in first year of life % *	55.5	91.2	77.9	80.3	80.3	54.4	4.0	18.6	**	46.3
Frequent URTIs (≥ quarterly) in second year of life % *	56.7	91.6	65.1	81.0	90.2	47.8	3.4	22.2	68.8	59.2
Frequent LRTIs (≥ quarterly) in first year of life % *	5.4	20.9	5.5	1.3	4.6	3.4	0.3	6.0	**	0.8
Frequent LRTIs (≥ quarterly) in second year of life % *	9.0	23.2	5.8	1.1	6.0	1.0	1.1	5.8	28.6	23.3
ALLERGIC DISEASE										
Eczema in first two years of life % *	34.7	53.0	56.0	46.1	37.4	33.1	4.9	26.7	**	18.7

Study centre (n) = total number of infants recruited. URTIs= Upper respiratory tract infections, LRTIs= Lower respiratory tract infections.

*p <0.05 using Chi-squared to test differences between centres.

# p<0.05 using Kruskal-Wallis to test differences between centres.

$ p<0.05 using one-way ANOVA to test differences between centres.

**For Milan, no one- year outcomes or variables dependent on these are specified because one-year data was not available for most participants.

4.4 Risk Factors for Wheeze in the Second Year of Life

4.4.1 Main Findings

4.4.1.1 Food Allergy

21.5% of infants diagnosed with food allergy had wheeze in the second year of life compared to 7.6% of infants without food allergy. Although food allergy was associated with an increased risk of wheeze in the second year of life (raw IRR 2.84, 95% CI 1.92-4.20, p <0.001 (Table 10), this association was not consistent across centres (Table 12Table 10) and was not significant after adjusting for potential confounders (adjusted IRR 1.26, 95% CI 0.55-2.91, p=0.589) (Table 10).

4.4.1.2 Feeding Practices

In univariate analysis any breastfeeding, longer duration of breastfeeding, increased overlap of breastfeeding/solids and later introduction of solids were associated with a lower prevalence of wheeze in some centres (Table 12). Any breastfeeding was significant in Reykjavik (raw IRR 0.41, 95% CI 0.19-0.86, p=0.019) and Lodz (raw IRR 0.33, 95% CI 0.12-0.89, p=0.028), duration of breastfeeding was significant in Reykjavik (raw IRR 0.95, 95% CI 0.91-0.99, p=0.022), age at introduction of solids was significant in Lodz (raw IRR 0.50, 95% CI 0.35-0.73, p <0.001) and overlap of breastfeeding/ solids was significant in Reykjavik (raw IRR 0.92, 95% CI 0.87-0.98, p=0.014) and Southampton (raw IRR 0.87, 95% CI 0.78-0.98, p=0.020) (Table 12). However, in the primary multivariable model, none of these factors were protective against wheeze (Table 10). Increased overlap of breastfeeding/solids showed a small protective effect in sensitivity model one (adjusted IRR 0.95, 95% CI 0.90-1.00) (Table 10).

4.4.1.3 Smoke Exposure

Maternal smoking ever was associated with a higher prevalence of wheeze in the second year of life (raw IRR 1.29, 95% CI 1.11-1.50, p=0.001), whilst having other smokers in the household was associated with a lower prevalence of wheeze (raw IRR 0.81, 95% CI 0.66-0.98, p=0.033). Neither of these factors were independently associated with wheeze in the second year of life (Table 10). Maternal smoking at one-year follow up was, however, a statistically significant risk factor for wheeze in multivariable analysis (adjusted IRR 1.62, 95% CI 1.09-2.42, p=0.017) (Table 10). It was a particularly strong risk factor in Southampton (adjusted IRR 2.72, 95% CI 1.29-5.77, p=0.009) (Table 13). Antenatal smoke exposure was not significant in univariate or multivariable analysis.

4.4.1.4 Other Potential Risk Factors

Factors associated with wheeze in the second year of life included male gender (raw IRR 1.35, 95% CI 1.16-1.58, p <0.001), higher birth weight (raw IRR 1.24, 95% CI 1.07-1.44, p=0.004), eczema (raw IRR 2.41, 95% CI 2.03-2.85, p <0.001), a family history of allergic disease, day care attendance (raw IRR 3.51, 95% CI 2.82-4.38, p <0.001) and frequent (≥ quarterly) respiratory tract infections. Longer birth length (raw IRR 0.96, 95% CI 0.94-0.99, p=0.004) and dog ownership (raw IRR 0.63, 95% CI 0.49-0.80, p <0.001) were associated with a lower prevalence of wheeze (Table 10). After adjusting for potential confounders, only male gender (adjusted IRR 1.33, 95% CI 1.03-1.70, p=0.027), maternal smoking at one-year follow up (adjusted IRR 1.62, 95% CI 1.09-2.42, p=0.017), day care attendance (adjusted IRR 1.63, 95% CI 1.08-2.45, p=0.020), frequent lower respiratory tract infections (LRTIs) in the first year of life (adjusted IRR 1.87, 95% CI 1.33-2.64, p<0.001) and frequent LRTIs in the second year of life (adjusted IRR 2.50, 95% CI 1.83-3.41, p<0.001) were statistically significant risk factors for wheeze (Table 10).

Table 10 Risk Factors for Wheeze in the Second Year of Life

	Unexposed % (n/N)	**Exposed** % (n/N)	**Wheeze in unexposed** % (n/N)	**Wheeze in exposed** % (n/N)	**Unadjusted IRR [95% CI] (p-value)**	**Primary model-Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 1-Adjusted IRR [95% CI] (p-value)** (n=4227)
FOOD ALLERGY							
Food allergy diagnosed in first two years of life (yes vs no)	98.6 (8654/8775)	1.4 (121/8775)	7.6 (655/8564)	21.5 (26/121)	2.84 [1.92-4.20] (<0.001)	1.26 [0.55-2.91] (0.589)	
FEEDING							
Ever breastfed (yes vs no)	8.8 (731/7607)	91.2 (7607/8338)	6.0 (44/731)	7.9 (597/7607)	1.30 [0.96-1.77] (0.089)	0.67 [0.14-3.18] (0.615)	
Duration of breastfeeding (per month increase)					1.00 [0.98-1.02] (0.918)	1.02 [0.92-1.12] (0.729)	
Age at introduction of first solids (per month increase)					0.98 [0.93-1.03] (0.384)	0.94 [0.83-1.08] (0.410)	
Overlap of breastfeeding/solids (per month increase)					0.99[0.96-1.03] (0.709)	0.94 [0.82-1.07] (0.320)	0.95 [0.90-1.00] (0.044)
SMOKE EXPOSURE							
Mother ever smoked (yes vs no)	59.8 (5243/8774)	40.2 (3531/8774)	7.0 (365/5243)	9.0 (316/3531)	1.29 [1.11-1.50] (0.001)	1.06 [0.81-1.40] (0.673)	
Smoking at any time during pregnancy (yes vs no)	91.8 (7843/8544)	8.2 (701/8544)	7.7 (601/7843)	9.6 (67/701)	1.25 [0.97-1.61] (0.086)	0.66 [0.40-1.10] (0.112)	
Mother smoking at one year follow up (yes vs no)	84.6 (5894/6971)	15.5 (1077/6971)	6.9 (404/5894)	7.9 (85/1077)	1.15 [0.91-1.45] (0.237)	1.62 [1.09-2.42] (0.017)	1.45 [1.09-1.92] (0.011)
Other smokers in household (yes vs no)	79.1 (6936/8774)	21.0 (1838/8774)	8.1 (561/6936)	6.5 (120/1838)	0.81 [0.66-0.98] (0.033)	1.25 [0.85-1.85] (0.261)	

	Unexposed % (n/N)	**Exposed** % (n/N)	**Wheeze in unexposed** % (n/N)	**Wheeze in exposed** % (n/N)	**Unadjusted IRR [95% CI] (p-value)**	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=4227)
BASIC DEMOGRAPHICS AND BIRTH DETAILS							
Male gender (vs female)	48.6 (4263/8774)	51.4 (4511/8774)	6.6 (280/4263)	8.9 (401/4511)	1.35 [1.16-1.58] (<0.001)	1.33 [1.03-1.70] (0.027)	1.32 [1.06-1.64] (0.014)
Gestation (per week increase)					1.04 [0.99-1.10] (0.105)	1.02 [0.93-1.12] (0.700)	
Birth weight (per kg increase)					1.24 [1.07-1.44] (0.004)	0.88 [0.60-1.28] (0.495)	
Birth length (per cm increase)					0.96 [0.94-0.99] (0.004)	0.99 [0.92-1.06] (0.715)	
Apgar score at 5 mins (per 1 point increase)					0.94 [0.84-1.053] (0.282)		
Multiple birth (vs single birth)	97.9 (8577/8760)	2.1 (183/8760)	7.7 (663/8577)	8.7 (16/183)	1.13 [0.69-1.86] (0.626)		
Caesarean delivery (vs vaginal delivery)	75.4 (6587/8731)	24.6 (2144/8731)	7.7 (506/6587)	8.1 (173/2144)	1.05 [0.88-1.25] (0.577)		
Non-Caucasian mother (vs Caucasian mother)	95.4 (8330/8734)	4.6 (404/8734)	7.7 (640/8330)	9.9 (40/404)	1.29 [0.94-1.77] (0.120)		
Non-Caucasian father (vs Caucasian father)	94.8 (8255/8708)	5.2 (453/8708)	7.5 (622/8255)	11.9 (54/453)	1.58 [1.20-2.09] (0.001)	1.44 [0.85-2.44] (0.180)	
Maternal age (per 1 year increase)					1.00 [0.98-1.01] (0.691)		
Paternal age, years (per 1 year increase)					1.00 [0.99-1.01] (0.918)		
FAMILIAL ALLERGIC DISEASE							
Maternal self-reported, doctor-diagnosed allergic disease							
-Any (yes vs no)	73.7 (6435/8732)	26.3 (2297/8732)	6.0 (386/6435)	12.6 (290/2297)	2.11[1.81-2.45] (<0.001)	1.13 [0.84-1.52] (0.428)	
-Asthma (yes vs no)	90.8 (809/8760)	9.2 (809/8760)	6.8 (544/7951)	16.7 (135/809)	2.44 [2.02-2.95] (<0.001)	1.30 [0.90-1.87] (0.158)	1.47 [1.12-1.93] (0.006)

	Unexposed % (n/N)	**Exposed** % (n/N)	**Wheeze in unexposed** % (n/N)	**Wheeze in exposed** % (n/N)	**Unadjusted IRR [95% CI] (p-value)**	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=4227)
-Allergic rhinitis (yes vs no)	84.8 (7418/8749)	15.2 (1331/8749)	6.9 (511/7418)	12.5 (166/1331)	1.81 [1.52-2.16] (<0.001)		
-Eczema (yes vs no)	88.1 (7706/8748)	11.9 (1042/8748)	7.0 (537/7706)	13.4 (140/1042)	1.93 [1.60-2.32] (<0.001)		
Paternal self-reported, doctor-diagnosed allergic disease							
-Any (yes vs no)	78.7 (6811/8652)	21.3 (1841/8652)	6.3 (427/6811)	12.7 (234/1841)	2.03 [1.73-2.38] (<0.001)	1.32 [0.98-1.78] (0.067)	1.31 [1.04-1.65] (0.020)
-Asthma (yes vs no)	92.9 (8083/8699)	7.1 (616/8699)	7.1 (571/8083)	15.4 (95/616)	2.18 [1.76-2.71] (<0.001)	0.73 [0.46-1.15] (0.174)	
-Allergic rhinitis (yes vs no)	85.7 (7415/8657)	14.4 (1242/8657)	7.1 (528/7415)	11.2 (139/1242)	1.58 [1.30-1.90] (<0.001)		
-Eczema (yes vs no)	93.8 (8153/8688)	6.2 (535/8688)	7.2 (587/8153)	14.8 (79/535)	2.05 [1.62-2.60] (<0.001)		
LIVING ENVIRONMENT							
Rural housing (vs urban housing)	83.8 (7352/8774)	16.2 (1422/8774)	7.9 (580/7352)	7.1 (101/1422)	0.90 [0.73-1.11] (0.330)		
Mould in house (yes vs no)	90.2 (7667/8503)	9.8 (836/8503)	7.5 (573/7667)	9.5 (79/836)	1.26 [1.00-1.60] (0.051)	0.96 [0.64-1.44] (0.833)	
Pets							
-Any (yes vs no)	63.9 (5594/8751)	36.1 (3157/8751)	7.8 (438/5594)	7.6 (241/3157)	0.98 [0.83-1.14] (0.752)		
-Cat (yes vs no)	84.6 (7398/8751)	15.5 (1353/8751)	7.7 (566/7398)	8.4 (113/1353)	1.09 [0.89-1.34] (0.395)		
-Dog (yes vs no)	83.9 (7343/8751)	16.1 (1408/8751)	8.3 (606/7343)	5.2 (73/1408)	0.63 [0.49-0.80] (<0.001)	0.90 [0.60-1.33] (0.587)	

	Unexposed % (n/N)	**Exposed** % (n/N)	**Wheeze in unexposed** % (n/N)	**Wheeze in exposed** % (n/N)	**Unadjusted IRR [95% CI] (p-value)**	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=4227)
MATERNAL EDUCATION							
Basic not completed					1.16 [0.77-1.74] (0.483)		
Basic completed					Baseline comparator		
Junior College/ vocational					1.15 [0.91-1.45] (0.255)		
College/ university					1.20 [0.96-1.50] (0.114)		
DAY CARE ATTENDANCE							
Day care in first year of life (yes vs no)	69.1 (4780/6922)	30.9 (2142/6922)	5.0 (239/4780)	11.5 (247/2142)	2.31 [1.93-2.76] (<0.001)		
Day care in second year of life (yes vs no)	44.7 (3908/8739)	55.3 (4831/8739)	4.0 (154/3891)	10.8 (522/4820)	2.74 [2.29-3.28] (<0.001)		
Day care at any time in first two years of life (yes vs no)	38.3 (3051/7966)	61.7 (4915/7966)	3.1 (93/3051)	10.7 (526/4915)	3.51 [2.82-4.38] (<0.001)	1.63 [1.08-2.45] (0.020)	1.70 [1.18-2.45] (0.004)
RESPIRATORY TRACT INFECTIONS							
URTIs in first year of life (≥ quarterly vs ≤ one)	45.2 (3142/6956)	54.8 (3814/6956)	3.3 (102/3142)	10.2 (388/3814)	3.13 [2.52-3.90] (<0.001)	1.08 [0.75-1.56] (0.672)	
URTIs in second year of life (≥ quarterly vs ≤ one)	42.1 (3626/8604)	57.9 (4978/8604)	3.8 (136/3626)	10.8 (537/4978)	2.88 [2.38-3.47] (<0.001)	1.08 [0.72-1.62] (0.704)	
LRTIs in first year of life (≥ quarterly vs ≤ one)	94.7 (6524/6886)	5.3 (362/6886)	6.2 (406/6524)	22.4 (81/362)	3.60 [2.83-4.56] (<0.001)	1.87 [1.33-2.64] (<0.001)	1.72 [1.25-2.36] (0.001)
LRTIs in second year of life (≥ quarterly vs ≤ one)	91.0 7252/7967	9.0 715/7967	6.6 (479/7252)	24.9 (178/715)	3.77 [3.17-4.48] (<0.001)	2.50 [1.83-3.41] (<0.001)	2.36 [1.76-3.17] (<0.001)
ALLERGIC DISEASE							
Eczema in first two years of life (yes vs no)	67.7 (4870/7198)	32.3 (2328/7198)	5.1 (247/4870)	12.2 (284/2328)	2.41 [2.03-2.85] (<0.001)	1.20 [0.93-1.55] (0.158)	1.35 [1.08-1.69] (0.009)

	Unexposed % (n/N)	**Exposed** % (n/N)	**Wheeze in unexposed** % (n/N)	**Wheeze in exposed** % (n/N)	**Unadjusted IRR [95% CI] (p-value)**	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=4227)
STUDY CENTRE							
Reykjavik	87.8 (7700/8775)	1075/8775 (12.3)	6.4 (496/7700)	17.2 (185/1075)	Baseline comparator	Baseline comparator	Baseline comparator
Southampton	91.3 (8011/8775)	8.7 (764/8775)	7.3 (581/8011)	13.1 (100/764)	0.76 [0.60-0.97] (0.027)	1.04 [0.65-1.68] (0.866)	1.09 [0.74-1.60] (0.668)
Amsterdam	92.9 (8153/8775)	7.1 (622/8775)	7.5 (614/8153)	10.8 (67/622)	0.63 [0.47-0.83] (<0.001)	0.72 [0.36-1.41] (0.335)	0.82 [0.54-1.24] (0.351)
Berlin	85.3 (7482/8775)	14.7 (1293/8775)	7.1 (529/7482)	11.8 (152/1293)	0.68 [0.55-0.85] (<0.001)	0.98 [0.69-1.39] (0.912)	0.99 [0.73-1.35] (0.957)
Lodz	85.7 (7520/8775)	14.3 (1255/8775)	8.8 (660/7520)	1.7 (21/1255)	0.10 [0.06-0.15] (<0.001)	0.18 [0.08-0.42] (<0.001)	0.17 [0.08-0.36] (<0.001)
Vilnius	86.7 (7611/8775)	13.3 (1164/8775)	8.7 (659/7611)	1.9 (22/1164)	0.11 [0.07-0.17] (<0.001)	0.34 [0.15-0.78] (0.010)	0.33 [0.18-0.60] (<0.001)
Madrid	89.6 (7865/8775)	10.4 (910/8775)	8.3 (654/7865)	3.0 (27/910)	0.17 [0.13-0.26] (<0.001)	0.18 [0.08-0.39] (<0.001)	0.23 [0.13-0.41] (<0.001)
Milan	89.8 (7878/8775)	10.2 (897/8775)	7.6 (596/7878)	9.5 (85/897)	0.55 [0.43-0.71] (<0.001)	1.33 [0.17-10.20] (0.781)	2.67 [0.65-10.97] (0.172)
Athens	90.9 (7980/8775)	9.1 (795/8775)	8.3 (659/7980)	2.8 (22/795)	0.16 [0.10-0.25] (<0.001)	0.34 [0.05-2.61] (0.302)	0.33 [0.04-2.40] (0.272)

IRR= Incidence rate ratio.

URTIs= Upper respiratory tract infections, LRTIs= Lower respiratory tract infections.

Primary model: Includes all variables with p<0.1 in univariate analysis (gender, gestation, birth weight, birth length, ethnicity of father, maternal allergy, maternal asthma, paternal allergy, paternal asthma, mould in house, dog ownership, day care attendance, respiratory tract infections and eczema), plus food allergy, variables related to feeding and smoke exposure and study centre.

Sensitivity model 1: This was generated by applying backward deletion to the primary model. It includes overlap of breastfeeding/solids, mother smoking at one-year follow up, gender, day care attendance, lower respiratory tract infections, eczema and study centre.

4.4.2 Findings of Alternative Models

The risk factors for wheeze in the second year of life identified by the primary multivariable model (Table 10) were also identified by all three alternative multivariable models (Table 11). Additional risk factors identified by one or more of these included increased overlap of breastfeeding/solids, maternal asthma, paternal allergic disease, having a non-Caucasian father, frequent upper respiratory tract infections in the first and second years of life and eczema. Paternal allergic disease was a significant risk factor in all three alternative models, eczema was significant in the models generated using backward deletion (sensitivity models one and three) and upper respiratory tract infections and maternal asthma were significant in the models not adjusted for 'study centre' (sensitivity models two and three). Overlap of breastfeeding/solids was only significant in sensitivity model one and paternal ethnicity was only significant in sensitivity model three.

4.5 Risk Factors for Wheeze by Study Centre

The association between the risk factors identified in the primary model and wheeze in the second year of life varied across Europe (Table 13). In Reykjavik (Iceland), for example, only frequent lower respiratory tract infections in the first (adjusted IRR 1.83, 95% CI 1.28-2.65, p=0.001) and second years of life (adjusted IRR 2.74, 95% CI 1.92-3.92, p <0.001) were statistically significant risk factors for wheeze, whereas in Southampton (UK) only maternal smoking at one-year follow up was important (adjusted IRR 2.72, 95% CI 1.29-5.77, p=0.009).

4.6 Risk Factors for Recurrent Wheeze

The risk factors for recurrent wheeze (Table 14) and wheeze in the second year of life were largely similar (Table 10). A noteworthy finding is that maternal asthma and paternal allergic disease were significant in all four models for recurrent wheeze, including the primary model (adjusted IRR 1.81, 95% CI 1.09-3.01, p=0.022 for maternal asthma and adjusted IRR 1.57, 95% CI 1.02-2.41, p=0.039 for paternal allergic disease). In sensitivity models two and three for recurrent wheeze, increased birth length was protective (adjusted IRR 0.88, 95% CI 0.81-0.95, p=0.001 in sensitivity model three). Increased gestational age was also protective according to sensitivity model one (adjusted IRR 0.88, 95% CI 0.81-0.97, p=0.010) whilst food allergy was a statistically significant risk factor for wheeze according to sensitivity model three (IRR 2.38, 95% CI 1.22-4.63, p=0.011). Interestingly, day care was not a risk factor for recurrent wheeze.

Table 11 Alternative Multivariable Models to Evaluate Risk Factors for Wheeze in the Second Year of Life

	Unadjusted IRR [95% CI] (p-value)	**Sensitivity model 2 - Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4172)
FOOD ALLERGY			
Food allergy diagnosed in first two years of life (yes vs no)	2.84 (<0.001) [1.92-4.20]	1.29 [0.56-2.99] (0.547)	
FEEDING			
Ever breastfed (yes vs no)	1.30 (0.089) [0.96-1.77]	0.72 [0.15-3.39] (0.673)	
Duration of breastfeeding (per month increase)	1.00 (0.918) [0.98-1.02]	1.01 [0.92-1.12] (0.760)	
Age at introduction of first solids (per month increase)	0.98 (0.384) [0.93-1.03]	0.95 [0.83-1.08] (0.447)	
Overlap of breastfeeding/solids (per month increase)	0.99 (0.709) [0.96-1.03]	0.94 [0.83-1.07] (0.361)	
SMOKE EXPOSURE			
Mother ever smoked (yes vs no)	1.29 (0.001) [1.11-1.50]	1.15 [0.87-1.51] (0.322)	
Smoking at any time during pregnancy (yes vs no)	1.25 (0.086) [0.97-1.61]	0.66 [0.40-1.09] (0.105)	
Mother smoking at one year follow up (yes vs no)	1.15 (0.237) [0.91-1.45]	1.56 [1.05-2.30] (0.026)	1.41 [1.07-1.87] (0.015)
Other smokers in household (yes vs no)	0.81 (0.033) [0.66-0.98]	1.05 [0.72-1.53] (0.814)	
BASIC DEMOGRAPHICS AND BIRTH DETAILS			
Male gender (vs female)	1.35 [1.16-1.58] (<0.001)	1.31 [1.02-1.68] (0.032)	1.31 [1.05-1.63] (0.017)
Gestation (per week increase)	1.04 [0.99-1.10] (0.105)	1.02 [0.93-1.12] (0.680)	
Birth weight (per kg increase)	1.24 [1.07-1.44] (0.004)	1.02 [0.71-1.43] (0.955)	
Birth length (per cm increase)	0.96 [0.94-0.99] (0.004)	0.97 [0.91-1.03] (0.346)	
Apgar score at 5 minutes (per 1 point increase)	0.94 [0.84-1.05] (0.282)		
Multiple birth (vs single birth)	1.13 [0.69-1.86] (0.626)		
Caesarean delivery (vs vaginal delivery)	1.05 [0.88-1.25] (0.577)		
Non-Caucasian mother (vs Caucasian mother)	1.29 [0.94-1.77] (0.120)		
Non-Caucasian father (vs Caucasian father)	1.58 [1.20-2.09] (0.001)	1.37 [0.82-2.29] (0.232)	1.50 [1.01-2.21] (0.043)

	Unadjusted IRR [95% CI] (p-value)	**Sensitivity model 2 - Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4172)
Maternal age (per 1 year increase)	1.00 [0.98-1.01] (0.691)		
Paternal age (per 1 year increase)	1.00 [0.99-1.01] (0.918)		
FAMILIAL ALLERGIC DISEASE			
Maternal self-reported, doctor-diagnosed allergic disease			
-Any (yes vs no)	2.11 [1.81-2.45] (<0.001)	1.28 [0.95-1.71] (0.105)	
-Asthma (yes vs no)	2.44 [2.02-2.95] (<0.001)	1.36 [0.94-1.94] (0.104)	1.71 [1.30-2.24] (<0.001)
-Allergic rhinitis (yes vs no)	1.81 [1.52-2.16] (<0.001)		
-Eczema (yes vs no)	1.93 [1.60-2.32] (<0.001)		
Paternal self-reported, doctor-diagnosed allergic disease			
-Any (yes vs no)	2.03 [1.73-2.38] (<0.001)	1.36 [1.01-1.83] (0.044)	1.40 [1.11-1.60] (0.004)
-Asthma (yes vs no)	2.18 [1.76-2.71] (<0.001)	0.76 [0.48-1.20] (0.244)	
-Allergic rhinitis (yes vs no)	1.572 [1.304-1.895] (<0.001)		
-Eczema (yes vs no)	2.051 [1.622-2.594] (<0.001)		
LIVING ENVIRONMENT			
Rural housing (vs urban housing)	0.90 [0.73-1.11] (0.330)		
Mould in house (yes vs no)	1.26 [1.00-1.60] (0.051)	1.01 [0.68-1.51] (0.954)	
Pets			
-Any (yes vs no)	0.98 [0.83-1.14] (0.752)		
-Cat (yes vs no)	1.09 [0.89-1.34] (0.395)		
-Dog (yes vs no)	0.63 [0.49-0.80] (<0.001)	0.82 [0.55-1.22] (0.322)	
DAY CARE ATTENDANCE			
Day care in first year of life (yes vs no)	2.31 [1.93-2.76] (<0.001)		
Day care in second year of life (yes vs no)	2.74 [2.29-3.28] (<0.001)		
Day care at any time in first two years of life (yes vs no)	3.51 [2.82-4.38] (<0.001)	2.32 [1.58-3.40] (<0.001)	2.52 [1.81-3.53] (<0.001)

	Unadjusted IRR [95% CI] (p-value)	**Sensitivity model 2 - Adjusted IRR [95% CI] (p-value)** (n=3612)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4172)
RESPIRATORY TRACT INFECTIONS			
URTIs in first year of life (≥ quarterly vs ≤ one)	3.13 [2.52-3.90] (<0.001)	1.55 [1.09-2.19] (0.014)	1.65 [1.21-2.26] (0.002)
URTIs in second year of life (≥ quarterly vs ≤ one)	2.88 [2.38-3.47] (<0.001)	1.62 [1.11-2.36] (0.012)	1.41 [1.02-1.93] (0.036)
LRTIs in first year of life (≥ quarterly vs ≤ one)	3.60 [2.84-4.56] (<0.001)	1.77 [1.27-2.46] (0.001)	1.69 [1.24-2.30] (0.001)
LRTIs in second year of life (≥ quarterly vs ≤ one)	3.77 [3.17-4.48] (<0.001)	2.51 [1.85-3.41] (<0.001)	2.45 [1.84-3.26] (<0.001)
ALLERGIC DISEASE			
Eczema in first two years of life (yes vs no)	2.41 [2.03-2.85] (<0.001)	1.21 [0.94-1.55] (0.147)	1.36 [1.09-1.70] (0.007)

IRR= Incidence rate ratio

LRTIs= Lower respiratory tract infections, URTIs= Upper respiratory tract infections

Sensitivity model 2: Includes all variables with p<0.1 in univariate analysis (gender, gestation, birth weight, birth length, ethnicity of father, maternal allergy, maternal asthma, paternal allergy, paternal asthma, mould in house, dog ownership, day care attendance, respiratory tract infections and eczema) plus food allergy and variables related to feeding and smoke exposure.

Sensitivity model 3: This was generated by applying backward deletion to sensitivity model 2. It includes mother smoking at one-year follow up, gender, ethnicity of father, maternal asthma, paternal allergy, day care attendance, respiratory tract infections and eczema.

Table 12 Risk Factors for Wheeze in the Second Year of Life by Centre (Unadjusted)

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
FOOD ALLERGY										
Food allergy diagnosed in first two years of life (yes vs no)	2.84 [1.92-4.20] (<0.001)	2.07 [1.02-4.20] (0.045)	3.34 [1.68-6.62] (0.001)	0.98 [0.24-3.99] (0.974)	1.22 [0.30-4.92] (0.782)	3.87 [0.52-28.85] (0.186)	-	8.63 [2.60-28.64] (<0.001)	1.51 [0.21-10.87] (0.680)	-
FEEDING										
Ever breastfed (yes vs no)	1.30 [0.96-1.77] (0.089)	0.41 [0.19-0.86] (0.019)	1.20 [0.55-2.60] (0.643)	1.32 [0.65-2.68] (0.437)	1.97 [0.63-6.18] (0.246)	0.33 [0.12-0.89] (0.028)	-	1.27 [0.38-4.22] (0.695)	0.87 [0.43-1.73] (0.685)	3.86 [0.52-28.80] (0.187)
Duration of breastfeeding (per month increase)	1.00 [0.98-1.02] (0.918)	0.95 [0.91-0.99] (0.022)	0.93 [0.87-0.98] (0.014)	0.97 [0.89-1.05] (0.421)	0.98 [0.93-1.03] (0.453)	0.90 [0.77-1.04] (0.148)	0.97 [0.85-1.11] (0.653)	1.01 [0.87-1.18] (0.879)	0.96 [0.89-1.03] (0.253)	1.03 [0.89-1.20] (0.658)
Age at introduction of first solids (per month increase)	0.98 [0.93-1.03] (0.384)	0.95 [0.85-1.07] (0.409)	1.05 [0.92-1.20] (0.489)	0.99 [0.85-1.16] (0.903)	0.91 [0.82-1.01] (0.065)	0.50 [0.35-0.73] (<0.001)	1.02 [0.88-1.19] (0.785)	1.10 [0.93-1.31] (0.279)	1.02 [0.89-1.17] (0.770)	1.26 [0.90-1.77] (0.173)
Overlap of breast feeding/solids (per month increase)	0.99 [0.96-1.03] (0.709)	0.92 [0.87-0.98] (0.014)	0.87 [0.78-0.98] (0.020)	0.97 [0.84-1.12] (0.654)	0.98 [0.90-1.07] (0.666)	0.89 [0.71-1.11] (0.290)	0.93 [0.74-1.16] (0.515)	1.06 [0.87-1.29] (0.574)	0.92 [0.82-1.03] (0.162)	1.05 [0.85-1.28] (0.670)
SMOKE EXPOSURE										
Mother ever smoked (yes vs no)	1.29 [1.11-1.49] (0.001)	1.00 [0.74-1.35] (0.995)	1.06 [0.71-1.59] (0.767)	1.36 [0.84-2.20] (0.207)	1.41 [1.02-1.95] (0.038)	0.68 [0.25-1.86] (0.457)	1.55 [0.67-3.58] (0.302)	1.86 [0.87-3.97] (0.110)	1.52 [0.99-2.33] (0.054)	1.41 [0.61-3.27] (0.419)
Smoking at any time during pregnancy (yes vs no)	1.25 [0.97-1.61] (0.086)	1.53 [0.96-2.44] (0.071)	2.28 [1.00 -5.20] (0.051)	1.62 [0.85-3.10] (0.142)	0.68 [0.35-1.33] (0.262)	2.30 [0.68-7.80] (0.182)	1.68 [0.39-7.21] (0.482)	0.81 [0.19-3.48] (0.781)	1.76 [0.88-3.52] (0.109)	1.48 [0.54-4.05] (0.441)
Mother smoking at one year follow up (yes vs no)	1.15 [0.91-1.45] (0.237)	1.22 [0.72-2.05] (0.464)	2.60 [1.21-5.44] (0.011)	1.49 [0.71-2.87] (0.236)	1.20 [0.79-1.82] (0.390)	2.16 [0.70-6.69] (0.183)	0.67 [0.09-5.01] (0.695)	1.45 [0.63-3.34] (0.379)	3.17 [0.33- 30.44] (0.318)	1.62 [0.67-3.19] (0.283)
Other smokers in household (yes vs no)	0.81 [0.66-0.98] (0.033)	1.13 [0.50-2.55] (0.770)	1.83 [1.14-2.94] (0.012)	1.20 [0.67-2.17] (0.539)	1.20 [0.70-2.05] (0.502)	2.11 [0.90-4.97] (0.088)	1.73 [0.75-4.01] (0.198)	0.98 [0.40-2.58] (0.960)	1.48 [0.94-2.33] (0.094)	1.56 [0.67-3.66] (0.303)

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
BASIC DEMOGRAPHICS AND BIRTH DETAILS										
Male gender (vs female)	1.35 [1.16-1.58] (<0.001)	1.28 [0.95-1.71] (0.101)	1.39 [0.93-2.08] (0.104)	2.36 [1.38-4.05] (0.002)	1.21 [0.86-1.66] (0.253)	1.53 [0.63-3.68] (0.346)	1.70 [0.71-4.03] (0.236)	1.07 [0.50-2.28] (0.856)	1.32 [0.86-2.03] (0.200)	0.89 [0.39-2.05] (0.781)
Gestation (per week increase)	1.04 [0.99-1.10] (0.105)	0.89 [0.81-0.98] (0.012)	1.02 [0.89-1.16] (0.783)	0.88 [0.75-1.03] (0.105)	1.04 [0.93-1.16] (0.516)	1.05 [0.80-1.37] (0.752)	0.82 [0.60-1.12] (0.200)	1.08 [0.83-1.41] (0.583)	0.93 [0.82-1.05] (0.225)	1.05 [0.78-1.42] (0.758)
Birth weight (per kg increase)	1.24 [1.07-1.44] (0.004)	0.85 [0.64-1.13] (0.257)	1.15 [0.78-1.69] (0.482)	0.72 [0.45-1.13] (0.152)	1.08 [0.78-1.49] (0.654)	0.70 [0.30-1.62] (0.402)	0.60 [0.24-1.56] (0.296)	2.07 [0.86-5.00] (0.106)	0.83 [0.55-1.25] (0.376)	0.53 [0.21-1.32] (0.170)
Birth length (per cm increase)	0.96 [0.94-0.99] (0.004)	0.97 [0.91-1.03] (0.326)	0.94 [0.87-1.00] (0.052)	0.89 [0.72-1.10] (0.276)	1.02 [0.96-1.09] (0.493)	0.93 [0.82-1.05] (0.235)	0.94 [0.79-1.13] (0.511)	1.15 [0.95-1.40] (0.145)	0.99 [0.91-1.08] (0.845)	0.95 [0.84-1.07] (0.390)
Apgar score at 5 mins (per 1 point increase)	0.94 [0.84-1.05] (0.282)	1.01 [0.84-1.22] (0.907)	1.07 [0.727-1.57] (0.741)	0.81 [0.54-1.22] (0.310)	0.95 [0.76-1.12] (0.675)	0.67 [0.35-1.27] (0.218)	1.19 [0.44-3.21] (0.739)	0.49 [0.26-0.93] (0.028)	0.98 [0.66-1.45] (0.922)	0.84 [0.28-2.52] (0.751)
Multiple birth (vs single birth)	1.13 [0.69-1.86] (0.626)	1.59 [0.51-4.99] (0.423)	1.09 [0.27-4.25] (0.902)	0.93 [0.13-6.68] (0.940)	0.61 [0.20-1.93] (0.403)	2.29 [0.53-9.84] (0.264)	-	-	3.65 [1.40-8.56] (0.007)	-
Caesarean delivery (vs vaginal delivery)	1.05 [0.88-1.25] (0.577)	1.34 [0.91-1.98] (0.133)	1.11 [0.73-1.69] (0.640)	0.56 [0.23-1.39] (0.213)	0.99 [0.70-1.39] (0.931)	1.02 [0.42-2.46] (0.969)	1.22 [0.41-3.61] (0.716)	1.55 [0.21-11.40] (0.668)	1.45 [0.94-2.25] (0.093)	1.47 [0.64-3.41] (0.365)
Non-Caucasian mother (vs Caucasian mother)	1.29 [0.94-1.77] (0.120)	-	0.73 [0.23-2.30] (0.587)	1.27 [0.74-2.19] (0.384)	0.75 [0.33-1.70] (0.490)	-	-	2.53 [1.07-5.99] (0.034)	1.53 [0.67-3.50] (0.317)	-
Non-Caucasian father (vs Caucasian father)	1.58 [1.20-2.09] (0.001)	1.37 [0.51-3.69] (0.533)	0.34 [0.052.45] (0.285)	1.37 [0.84-2.34] (0.245)	1.11 [0.63-1.95] (0.731)	-	-	2.98 [1.1-6.81] (0.010)	2.37 [1.19-4.74] (0.014)	-
Maternal age (per 1 year increase)	1.00 [0.98-1.01] (0.691)	0.96 [0.93-0.99] (0.008)	0.99 [0.94-1.03] (0.480)	0.99 [0.94-1.04] (0.624)	0.98 [0.95-1.01] (0.224)	0.96 [0.86-1.06] (0.413)	1.08 [1.00-1.17] (0.057)	0.90 [0.84-0.96] (0.003)	0.99 [0.94-1.04] (0.710)	0.96 [0.88-1.06] (0.431)
Paternal age (per 1 year increase)	1.00 [0.99-1.01] (0.918)	0.98 [0.96-1.01] (0.186)	0.97 [0.93-1.01] (0.095)	0.99 [0.95-1.03] (0.564)	0.99 [0.96-1.02] (0.420)	0.95 [0.87-1.05] (0.327)	1.05 [0.99-1.12] (0.121)	0.94 [0.87-1.01] (0.087)	1.01 [0.97-1.05] (0.697)	1.01 [0.93- 1.09] (0.872)

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
FAMILIAL ALLERGIC DISEASE										
Maternal self-reported, doctor diagnosed allergic disease										
-Any (yes vs no)	2.11 [1.81-2.45] (<0.001)	1.55 [1.56 - 2.07] (0.003)	1.55 [1.04-2.32] (0.033)	1.32 [0.81-2.15] (0.264)	1.18 [0.85-1.64] (0.323)	1.65 [0.45-1.64] (0.423)	2.76 [0.82-9.33] (0.102)	0.50 [0.17-1.46] (0.206)	1.18 [0.73-1.89] (0.500)	1.69 [0.62-4.57] (0.304)
-Asthma (yes vs no)	2.44 [2.02-2.95] (<0.001)	1.85 [1.34-2.56] (<0.001)	1.70 [1.12-2.59] (0.014)	1.33 [0.69-2.53] (0.393)	1.26 [0.79-2.01] (0.343)	-	3.21 [0.43-23.89] (0.254)	1.91 [0.57-6.33] (0.292)	1.95 [1.01-3.46] (0.022)	2.70 [0.80-9.11] (0.111)
-Allergic rhinitis (yes vs no)	1.81 [1.52-2.16] (<0.001)	1.91 [1.38-2.65] (0.001)	1.17 [0.77-1.80] (0.463)	1.54 [0.919-2.590] (0.101)	1.18 [0.83-1.69] (0.359)	2.28 [0.67-7.79] (0.188)	4.33 [1.28-14.61] (0.018)	0.65 [0.20-2.16] (0.480)	1.16 [0.67-2.00] (0.597)	-
-Eczema (yes vs no)	1.93 [1.60-2.32] (<0.001)	1.11 [0.81-1.52] (0.515)	1.51 [1.01-2.27] (0.045)	1.24 [0.66-2.31] (0.502)	1.10 [0.70-1.73] (0.6760	-	-	-	1.29 [0.68-2.42] (0.438)	4.58 [1.07-19.58] (0.040)
Paternal self-reported, doctor diagnosed allergic disease										
-Any (yes vs no)	2.03 [1.73-2.38] (<0.001)	1.37 [1.01-1.85] (0.041)	1.66 [1.11-2.49] (0.013)	2.06 [1.26-3.37] (0.004)	0.97 [0.69-1.38] (0.880)	1.65 [0.49-5.60] (0.422)	5.08 [1.51-17.18] (0.009)	0.93 [0.35-2.46] (0.882)	1.46 [0.92-2.34] (0.112)	1.06 [0.31-3.57] (0.928)
-Asthma (yes vs no)	2.18 [1.76-2.71] (<0.001)	1.12 [0.70-1.78] (0.638)	1.39 [0.87-2.22] (0.172)	2.39 [1.30-4.39] (0.005)	1.47 [0.91-2.38] (0.118)	2.27 [0.31-16.94] (0.423)	10.48 [2.45-44.84] (0.002)	2.09 [0.63-6.98] (0.228)	2.00 [1.11-3.62] (0.021)	1.13 [0.15-8.42] (0.903)
-Allergic rhinitis (yes vs no)	1.57 [1.30-1.90] (<0.001)	1.07 [0.72-1.60] (0.740)	1.24 [0.79-1.94] (0.348)	1.99 [1.18-3.35] (0.010)	0.88 [0.60-1.29] (0.510)	2.05 [0.61-6.97] (0.249)	4.37 [1.02-18.71] (0.047)	0.93 [0.32-2.71] (0.898)	1.55 [0.9-2.56] (0.101)	0.95 [0.22-4.05] (0.940)
-Eczema (yes vs no)	2.05 [1.62-2.59] (<0.001)	1.44 [1.01-2.06] (0.047)	0.78 [0.42-1.42] (0.411)	1.47 [0.70-3.09] (0.307)	0.86 [0.45-1.63] (0.637)	-	-	-	2.31 [1.23-4.36] (0.010)	-
LIVING ENVIRONMENT										
Rural housing (yes vs no)	0.90 [0.73-1.11] (0.330)	0.98 [0.56-1.72] (0.943)	0.61 [0.40-0.93] (0.023)	-	1.14 [0.42-3.07] (0.800)	0.35 [0.08-1.50] (0.158)	0.62 [0.15-2.65] (0.517)	1.19 [0.41-3.44] (0.748)	0.56 [0.20-1.52] (0.254)	1.16 [0.27-4.97] (0.840)
Mould in house (yes vs no)	1.26 [1.00-1.60] (0.051)	0.46 [0.91-2.35] (0.117)	1.08 [0.58-2.02] (0.815)	1.34 [0.75-2.38] (0.318)	0.79 [0.44-1.39] (0.406)	6.17 [1.44-26.50] (0.014)	0.45 [0.06-3.33] (0.431)	1.80 [0.42-7.58] (0.428)	1.76 [0.95-3.24] (0.073)	0.80 [0.27-2.38] (0.693)
Pets										
-Any (yes vs no)	0.98 [0.83-1.14] (0.752)	0.94 [0.68-1.30] (0.705)	0.84 [0.56-1.24] (0.373)	0.78 [0.48-1.25] (0.300)	1.29 [0.93-1.79] (0.121)	0.72 [0.30-1.73] (0.458)	1.27 [0.55-2.92] (0.579)	1.24 [0.54-2.83] (0.613)	0.99 [0.60-1.63] (0.964)	1.48 [0.59 -3.77] (0.415)
-Cat (yes vs no)	1.09 [0.89-1.34] (0.395)	1.21 [0.79-1.86] (0.386)	0.83 [0.53-1.30] (0.419)	0.60 [0.33-1.08] (0.090)	1.38 [0.93-2.06] (0.111)	0.56 [0.13-2.42] (0.441)	0.81 [0.27-2.37] (0.699)	1.13 [0.27-4.76] (0.870)	1.22 [0.63-2.36] (0.559)	1.35 [0.18-10.07] (0.767)
- Dog (yes vs no)	0.63 [0.49-0.80] (<0.001)	0.73 [0.43-1.26] (0.264)	0.87 [0.50-1.54] (0.641)	0.54 [0.22-1.33] (0.179)	0.87 [0.47-1.61] (0.664)	0.59 [0.22-1.60] (0.296)	2.05 [0.86-4.88] (0.106)	1.72 [0.70-4.26] (0.241)	0.85 [0.39-1.85] (0.683)	1.27 [0.38-4.29] (0.702)

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
Maternal education: Only basic education completed vs:										
Basic education not completed	1.16 [0.77-1.74] (0.483)	1.86 [0.29-4.93] (0.815)	-	2.39 [0.29-19.45] (0.414)	1.89 [0.92-3.91] (0.082)	2.79 [0.56-13.81] (0.209)	1.73 [0.16-19.10] (0.654)	0.88 [0.22-3.35] (0.860)	-	2.79 [0.75-10.40] (0.126)
Junior College/ vocational	1.15 [0.91-1.45] (0.255)	0.77 [0.50-1.18] (0.223)	0.58 [0.30-1.10] (0.094)	1.04 [0.46-2.38] (0.918)	1.24 [0.68-2.26] (0.480)	1.35 [0.41-4.41] (0.625)	1.53 [0.28-8.34] (0.625)	0.99 [0.39-2.48] (0.974)	0.50 [0.24-1.04] (0.064)	1.17 [0.34-4.04] (0.804)
College/ university	1.20 [0.96-1.50] (0.114)	0.79 [0.53-1.16] (0.223)	0.52 [0.29-0.93] (0.028)	1.02 [0.45-2.32] (0.969)	1.15 [0.63-2.08] (0.656)	0.64 [0.22-1.83] (0.403)	2.27 [0.52-9.94] (0.275)	-	0.73 [0.37-1.43] (0.353)	1.20 [0.39-3.66] (0.751)
DAY CARE ATTENDANCE										
Day care in first year of life (yes vs no)	2.31 [1.93-2.76] (<0.001)	1.15 [0.80-1.64] (0.445)	1.28 [0.78-2.07] (0.327)	1.12 [0.61-2.05] (0.709)	1.45 [1.05-2.01] (0.024)	2.05 [0.47-9.01] (0.343)	-	0.63 [0.28-1.45] (0.280)	0.96 [0.16-5.76] (0.967)	-
Day care in second year of life (yes vs no)	2.74 [2.29-3.28] (<0.001)	1.29 [0.66-2.51] (0.464)	1.10 [0.74-1.65] (0.630)	1.44 [0.69-3.01] (0.335)	1.53 [0.95-2.44] (0.078)	1.46 [0.53-4.02] (0.463)	3.22 [1.39-7.45] (0.006)	0.78 [0.36-1.70] (0.535)	1.49 [0.93-2.39] (0.100)	0.79 [0.23-2.67] (0.702)
Day care at any time in first two years of life (yes vs no)	3.51 [2.82- 4.38] (<0.001)	1.96 [0.63-6.14] (0.247)	1.12 [0.69-1.82] (0.649)	1.62 [0.65-4.03] (0.301)	1.70 [1.02-2.81] (0.040)	1.73 [0.59-5.05] (0.319)	3.44 [1.41-8.43] (0.007)	0.70 [0.32-1.52] (0.361)	0.53 [0.07-3.85] (0.534)	0.83 [0.24-2.84] (0.762)
RESPIRATORY TRACT INFECTIONS										
URTIs in first year of life (≥ quarterly vs ≤ one)	3.13 [2.52-3.90] (<0.001)	1.32 [0.67-2.60] (0.418)	1.11 [0.60-2.03] (0.744)	3.07 [1.11-8.47] (0.031)	1.08 [0.71-1.66] (0.708)	0.66 [0.25-1.77] (0.405)	8.83 [3.18-24.52] (<0.001)	3.70 [1.71-8.00] (0.001)	0.67 [0.11-3.99] (0.657)	1.20 [0.50-2.87] (0.690)
URTIs in second year of life (≥ quarterly vs ≤ one)	2.88 [2.38-3.47] (<0.001)	1.41 [0.77-2.60] (0.267)	0.66 [0.44-0.97] (0.036)	1.49 [0.74-3.01] (0.267)	1.72 [0.88-3.38] (0.114)	0.68 [0.28-1.63] (0.384)	1.37 [0.18-10.19] (0.758)	5.12 [2.38-11.03] (<0.001)	3.71 [1.86-7.41] (<0.001)	0.95 [0.38-2.37] (0.915)
LRTIs in first year of life (≥ quarterly vs ≤ one)	3.60 [2.83-4.56] (<0.001)	2.47 [1.74-3.50] (<0.001)	1.83 [0.84-4.01] (0.130)	2.60 [0.63-10.64] (0.185)	1.30 [0.64-2.65] (0.472)	4.54 [1.03-19.97] (0.045)	19.02 [2.54-142.46] (0.004)	9.51 [4.31-20.94] (<0.001)	-	--
LRTIs in second year of life (≥ quarterly vs ≤ one)	3.77 [3.17-4.48] (<0.001)	2.92 [2.19-3.90] (<0.001)	2.04 [1.09-3.82] (0.026)	2.70 [0.66-11.02] (0.167)	1.25 [0.68-2.31] (0.473)	24.24 [8.16-72.02] (<0.001)	4.21 [0.57-31.32] (0.160)	15.11 [7.10-32.14] (<0.001)	3.33 [2.15-5.16] (<0.001)	6.60 [0.60-72.79] (0.123)

	All centres (n=12049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
ALLERGIC DISEASE										
Eczema in first two years of life (yes vs no)	2.41[2.03-2.85] (<0.001)	1.63 [1.17-2.28] (0.004)	1.54 [0.95-2.48] (0.078)	2.48 [1.48-4.16] (0.001)	1.36 [0.98-1.89] (0.063)	2.62 [1.03-6.63] (0.043)	1.18 [0.16-8.85] (0.871)	2.23 [1.02-4.86] (0.043)		1.49 [0.55-4.07] (0.437)

Values represent: Incidence rate ratio, IRR [95% confidence intervals] (p-value)

Where no figures are entered, Poisson estimation was not possible.

Table 13 Risk Factors for Wheeze in the Second Year of Life by Centre (Adjusted)

	Reykjavik	Southampton	Amsterdam	Berlin	Lodz	Vilnius	Madrid	Milan	Athens
n	829	494	569	1228	1084	1009	826		
Male gender (vs female)	1.45 [1.02-2.08] (0.040)	1.66 [1.00-2.76] (0.050)	3.17 [1.67-6.01] (<0.001)	1.72 [0.84-1.63] (0.346)	1.28 [0.45-3.65] (0.643)	2.00 [0.74-5.32] (0.167)	1.02 [0.46-2.25] (0.966)	-	-
Mother smoking at one-year follow up (yes vs no)	1.08 [0.64-1.83] (0.776)	2.72 [1.29-5.77] (0.009)	1.46 [0.75-2.86] (0.267)	1.27 [0.84-1.94] (0.258)	2.38 [0.75-7.55] (0.141)	0.79 [0.11-5.99] (0.823)	1.66 [0.68-4.05] (0.261)	-	-
Day care at any time in first two years of life (yes vs no)	1.30 [0.41-4.09] (0.656)	1.16 [0.69-1.95] (0.570)	1.71 [0.67-4.34] (0.258)	1.67 [1.01-2.77] (0.047)	1.91 [0.65-5.61] (0.237)	3.66 [1.47-9.13] (0.005)	0.69 [0.31-1.53] (0.365)	-	-
LRTIs in first year of life (≥ quarterly vs ≤ one)	1.83 [1.28-2.65] (0.001)	1.85 [0.82-4.17] (0.138)	2.33 [0.56-9.63] (0.243)	1.30 [0.63-2.66] (0.478)	2.48 [0.32-19.18] (0.384)	24.9 [2.98-207.18] (0.003)	2.42 [0.86-6.80] (0.093)	-	-
LRTIs in second year of life (≥ quarterly vs ≤ one)	2.74 [1.92-3.92] (<0.001)	1.07 [0.41-2.75] (0.896)	2.67 [0.65-11.15] (0.174)	1.29 [0.69-2.39] (0.421)	10.23 [1.34-78.33] (0.025)	-	11.83 [4.27-32.78] (<0.001)	-	-

Values represent: Adjusted incidence rate ratio, IRR [95% confidence intervals] (p-value).

Where no figures are entered, Poisson estimation was not possible.

Only significant associations from the primary model (gender, mother smoking at one-year follow up, day care attendance and LRTIs in the first and second years of life) were entered into the multivariable models for individual centres.

LRTIs= Lower respiratory tract infections.

Table 14 Risk Factors for Recurrent Wheeze (Wheeze in the First and Second Years of Life)

	Unadjusted IRR [95% CI] (p-value)	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=5044)	**Sensitivity model 2- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4688)
FOOD ALLERGY					
Food allergy diagnosed in first two years of life (yes vs no)	5.50 [3.30-9.14] (<0.001)	2.20 [0.77-6.23] (0.140)		2.61 [0.92-7.42] (0.071)	2.38 [1.22-4.63] (0.011)
FEEDING					
Ever breastfed (yes vs no)	1.24 [0.74-2.06] (0.416)	3.39 [0.34-33.28] (0.295)		3.81 [0.39-37.60] (0.252)	2.09 [1.00-4.35] (0.049)
Duration of breastfeeding (per month increase)	1.01 [0.98-1.05] (0.481)	0.98 [0.85-1.13] (0.799)		0.96 [0.83-1.23] (0.627)	
Age at introduction of first solids (per month increase)	0.93 [0.84-1.03] (0.163)	0.90 [0.74-1.11] (0.329)		0.90 [0.74-1.10] (0.300)	0.82 [0.72-0.92] (0.001)
Overlap of breastfeeding/ solids (per month increase)	1.02 [0.99-1.07] (0.211)	0.97 [0.80-1.18] (0.764)		1.01 [0.83-1.12] (0.903)	
SMOKE EXPOSURE					
Mother ever smoked (yes vs no)	1.20 [0.92-1.57] (0.177)	1.38 [0.93-2.07] (0.112)		1.39 [0.94-2.06] (0.102)	
Smoking at any time during pregnancy	1.16 [0.74-1.82] (0.519)	0.74 [0.34-1.59] (0.435)		0.78 [0.37-1.65] (0.510)	
Mother smoking at one year follow up (yes vs no)	1.05 [0.73-1.51] (0.795)	1.28 [0.66-2.48] (0.470)		1.16 [0.61-2.19] (0.654)	1.41 [1.07-1.87] (0.015)
Other smokers in household (yes vs no)	0.60 [0.41-0.88] (0.009)	1.64 [0.92-2.91] (0.094)		1.29 [0.73-2.25] (0.378)	

	Unadjusted IRR [95% CI] (p-value)	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=5044)	**Sensitivity model 2- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4688)
BASIC DEMOGRAPHIC AND BIRTH DETAILS					
Male gender (vs female)	2.13 [1.60-2.85] (<0.001)	1.92 [1.29-2.84] (0.001)	2.04 [1.49-2.78] (<0.001)	1.84 [1.35-2.72] (0.002)	1.77 [1.26-2.49] (0.001)
Gestation (per week increase)	1.08 [0.99-1.18] (0.097)	0.93 [0.81-1.07] (0.325)	0.88 [0.81-0.97] (0.010)	0.98 [0.85-1.13] (0.827)	
Birth weight (per kg increase)	1.73 [1.32-2.26] (<0.001)	1.16 [0.67-1.99] (0.600)		1.62 [0.97-2.70] (0.064)	1.84 [1.23-2.75] (0.003)
Birth length (per cm increase)	0.96 [0.92-1.01] (0.128)	0.97 [0.88-1.08] (0.622)		0.91 [0.83-1.00] (0.047)	0.88 [0.81-0.95] (0.001)
Apgar score at 5 mins (per 1 point increase)	0.83 [0.68-1.01] (0.061)	0.85 [0.66-1.09] (0.194)		0.82 [0.65-1.05] (0.116)	
Multiple birth (vs single birth)	0.61 [0.20-1.91] (0.396)				
Caesarean delivery (vs vaginal delivery)	0.70 [0.49-0.99] (0.043)	0.88 [0.52-1.50] (0.651)		0.75 [0.45-1.26] (0.277)	
Non-Caucasian mother (vs Caucasian mother)	1.33 [0.76-2.33] (0.315)				
Non-Caucasian father (vs Caucasian father)	1.86 [1.18-2.95] (0.008)	1.76 [0.81-3.82] (0.154)		1.60 [0.76-3.38] (0.216)	
Maternal age (per 1 year increase)	0.98 [0.96-1.01] (0.171)				
Paternal age (per 1 year increase)	0.99 [0.97-1.01] (0.403)				

	Unadjusted IRR [95% CI] (p-value)	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=5044)	**Sensitivity model 2- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4688)
FAMILIAL ALLERGIC DISEASE					
Maternal self-reported, doctor-diagnosed allergic disease					
-Any (yes vs no)	3.35 [2.57-4.39] (<0.001)	1.11 [0.70-1.75] (0.656)		1.27 [0.80-2.01] (0.304)	
-Asthma (yes vs no)	4.29 [3.18-5.80] (<0.001)	1.81 [1.09-3.01] (0.022)	1.85 [1.32-2.59] (<0.001)	2.13 [1.29-3.51] (0.003)	2.31 [1.60-3.34] (<0.001)
-Allergic rhinitis (yes vs no)	2.39 [1.77-3.22] (<0.001)				
-Eczema (yes vs no)	3.12 [2.30-4.23] (<0.001)				
Paternal self-reported, doctor-diagnosed allergic disease					
-Any (yes vs no)	2.63 [1.99-3.48] (<0.001)	1.57 [1.02-2.41] (0.039)	1.42 [1.06-1.92] (0.019)	1.67 [1.09-2.57] (0.019)	1.42 [1.02-1.98] (0.036)
-Asthma (yes vs no)	2.94 [2.03-4.26] (<0.001)	0.79 [0.42-1.48] (0.461)		0.78 [0.42-1.45] (0.431)	
-Allergic rhinitis (yes vs no)	1.99 [1.44-2.74] (<0.001)				
-Eczema (yes vs no)	2.57 [1.71-3.88] (<0.001)				
LIVING ENVIRONMENT					
Rural housing (vs urban housing)	1.35 [0.96-1.89] (0.083)	0.84 [0.43-1.65] (0.611)		1.60 [0.96-2.65] (0.071)	
Mould in house (yes vs no)	1.09 [0.705-1.676] (0.706)				
Pets					
-Any (yes vs no)	0.97 [0.74-1.28] (0.841)				
-Cat (yes vs no)	1.15 [0.80-1.63] (0.453)				
-Dog (yes vs no	0.66 [0.44-1.00] (0.051)	0.73 [0.40-1.33] (0.300)		0.72 [0.39-1.32] (0.289)	

	Unadjusted IRR [95% CI] (p-value)	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=5044)	**Sensitivity model 2- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4688)
DAY CARE ATTENDANCE					
Day care in first year of life (yes vs no)	4.03 [3.07-5.30] (<0.001)				
Day care in second year of life (yes vs no)	3.92 [2.80-5.49] (<0.001)				
Day care at any time in first two years of life (yes vs no)	3.90 [2.77-5.50] (<0.001)	1.17 [0.64-2.14] (0.621)		1.72 [0.98-2.99] (0.057)	
RESPIRATORY TRACT INFECTIONS					
URTIs in first year of life (≥ quarterly vs ≤ one)	6.23 [4.21-9.21] (<0.001)	1.52 [0.80-2.87] (0.202)	1.92 [1.17-3.13] (0.009)	2.45 [1.34-4.48] (0.004)	3.11 [1.95-4.90] (<0.001)
URTIs in second year of life (≥ quarterly vs ≤ one)	3.64 [2.60-5.08] (<0.001)	1.26 [0.70-2.29] (0.446)		1.45 [0.81-2.60] (0.206)	
LRTIs in first year of life (≥ quarterly vs ≤ one)	9.48 [7.05-12.76] (<0.001)	3.34 [2.17-5.14] (<0.001)	3.36 [2.30-4.93] (<0.001)	3.70 [2.42-5.66] (<0.001)	3.97 [2.73-5.77] (<0.001)
LRTIs in second year of life (≥ quarterly vs ≤ one)	7.20 [5.31-9.77] (<0.001)	2.57 [1.66-3.96] (<0.001)	2.42 [1.63-3.59] (<0.001)	2.82 [1.85-4.30] (<0.001)	2.45 [1.67-3.60] (<0.001)

	Unadjusted IRR [95% CI] (p-value)	**Primary model- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 1- Adjusted IRR [95% CI] (p-value)** (n=5044)	**Sensitivity model 2- Adjusted IRR [95% CI] (p-value)** (n=3177)	**Sensitivity model 3- Adjusted IRR [95% CI] (p-value)** (n=4688)
ALLERGIC DISEASE					
Eczema in first two years of life (yes vs no)	3.52 [2.68-4.63] (<0.001)	1.36 [0.94-1.97] (0.105)	1.77 [1.32-2.38] (<0.001)	1.39 [0.95-2.01] (0.086)	1.90 [1.22-4.63] (<0.001)

IRR- Incidence rate ratio. URTIs= Upper respiratory tract infections, LRTIs= Lower respiratory tract infections.

Primary model: Includes all variables with p<0.1 in univariate analysis (gender, gestation, birth weight, birth length, Apgar score at 5 minutes, type of delivery, maternal allergy, maternal asthma, paternal allergy, paternal asthma, rural vs urban housing, dog ownership, day care attendance, respiratory tract infections and eczema) plus food allergy, variables related to feeding and smoke exposure and study centre.

Sensitivity model 1: This was generated by applying backward deletion to the primary model. It includes gender, gestation, maternal asthma, paternal allergy, URTIs in the first year of life, LRTIs in the first and second years of life, eczema and study centre.

Sensitivity model 2: Includes all variables with p<0.1 in univariate analysis (gender, gestation, birth weight, birth length, Apgar score at 5 minutes, type of delivery, maternal allergy, maternal asthma, paternal allergy, paternal asthma, rural vs urban housing, dog ownership, day care attendance, respiratory tract infections and eczema) plus food allergy and variables related to feeding and smoke exposure.

Sensitivity model 3: This was generated by applying backward deletion to sensitivity model 2. It includes food allergy , breastfeeding, age at introduction of solids, mother smoking at one-year follow up, gender, birth weight, birth length, maternal asthma, paternal allergy, URTIs the first year of life, LRTIs in the first and second years of life and eczema.

Chapter 5: UBIOPRED Results- Allergic Sensitisation and Allergic Disease

5.1 Participants

5.1.1 Paediatric Participants

As outlined in Figure 6, 298 children with asthma or wheeze were screened to recruit 282 participants. Of these, 99 had severe asthma, 49 had mild to moderate asthma, 81 had severe preschool wheeze and 54 had mild to moderate wheeze. The number of participants in each cohort with data available for analysis is outlined in Figure 6.

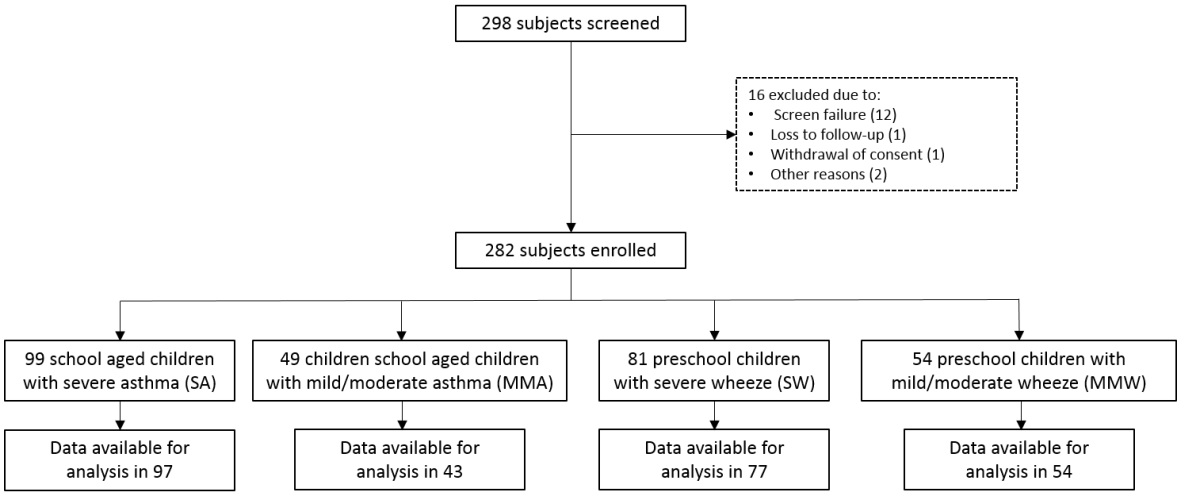

Figure 6 Consort Diagram for Paediatric UBIOPRED Participants

5.1.2 Adult Participants

Figure 7 shows that of 730 adults screened, 611 were enrolled in the study. This included 101 healthy controls, who were not included in this analysis. Of the 509 participants with asthma, 311 were non-smokers with severe asthma, 110 were current smokers or ex-smokers with severe asthma and 88 were non-smokers with mild to moderate asthma.

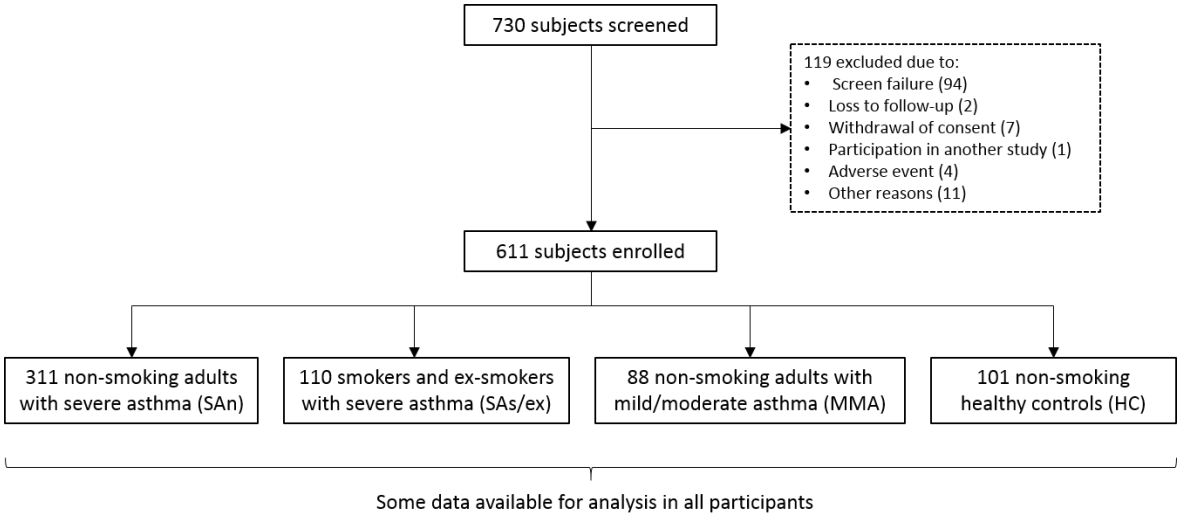

Figure 7 Consort Diagram for Adult UBIOPRED Participants

5.1.3 Baseline Characteristics of Participants in the Severe Cohorts

In the severe school aged asthma and smoking/ex-smoking adult cohorts, there were approximately equal numbers of males and females. In the severe preschool wheeze cohort, there were however more males (64.9%) and in the non-smoking adult cohort, there were more females (65.8%). The proportion of Caucasian participants was higher in the severe adult cohorts when compared to the severe paediatric cohorts. Most adult participants with severe disease were diagnosed in adult life (mean age of diagnosis 24.0 years for non-smokers and 33.6 years for smokers/ex-smokers). The proportion of participants admitted to ICU ever or in the past year was similar between the severe cohorts. However, the mean number of exacerbations in the past year was higher in preschool and school aged participants (3.9 in both groups compared to 2.5 and 2.6 in non-smoking adults and smokers/ex-smokers, respectively). Symptom triggers also differed between cohorts. Respiratory infections were a trigger in the majority of participants, particularly those with preschool wheeze (100%) and school aged participants with asthma (95%). Pollens and pets were more frequently a trigger for exacerbations in school aged participants. FEV_1 % predicted was lower in adults (mean 67.5 in non-smokers and 67.2 in smokers/ex-smokers) compared to preschool participants (mean 104.3) and school aged participants (mean 88.7) (Table 15).

5.1.4 Baseline Characteristics of Participants in the Mild to Moderate Cohorts

The proportion of female participants did not differ significantly between cohorts. There were, however, fewer Caucasian participants in the school aged cohort (74%) compared to the preschool (89%) and adult (93%) cohorts. The mean age of diagnosis for adult participants was 19.9 years. Once again, the proportion of participants admitted to ICU ever or on the past year did not differ between cohorts. Respiratory infections were a reported symptom trigger in 100% of preschool participants and 98% of school aged participants compared to only 70% of adults. As in the severe cohorts, pollens and pets were a more common symptom trigger in school aged participants with exercise being the most common trigger in adults (65%). FEV_1 % predicted did not differ between the cohorts but the mean FEV_1/FVC ratio was lower in adults (0.72 compared to 0.89 in preschool children and 0.80 in school aged children) (Table 15).

Table 15 Demographic Details and Asthma History of UBIOPRED Participants

	Severe Cohorts					Mild to Moderate Cohorts			
	Preschool children (SW)	**School aged children (SA)**	**Non-smoking adults (SAn)**	**Smokers and ex-smokers (SAs/ex)**	**p-value**	**Preschool children (SW)**	**School aged children (SA)**	**Non-smoking adults (SAn)**	**p-value**
n	77	97	311	110		54	43	88	
Demographic details									
Female	27/77 (35.1)	46/97 (47.4)	205/311 (65.9)	56/110 (50.9)	<0.001	20/54 (37.0)	16/43 (37.2)	44/88 (50.0)	0.210
Age (years)	3.56 ± 0.14 (n=77)	12.21 ± 0.31 (n=97)	51.01 ± 0.8 (n=311)	54.51 ± 1.08 (n=110)	<0.001	3.46 ± 0.16 (n=54)	11.26 ± 0.48 (n=43)	41.66 ± 1.65 (n=88)	<0.001
Caucasian	62/77 (80.5)	74/97 (76.3)	277/311 (89.1)	105/110 (95.5)	<0.001	48/54 (88.9)	32/43 (74.4)	82/88 (93.2)	0.009
Anthropometry									
Height (cm)	102.88 ± 1.13 (n=76)	152.82 ± 1.65 (n=97)	166.47 ± 0.01 (n=311)	169.21 ± 0.03 (n=110)	<0.001	103.62 ± 1.52 (n=53)	148.12 ± 2.58 (n=43)	170.88 ± 0.04 (n=88)	<0.001
Height z-score	1.14 ± 0.16 (n=76)	0.68 ± 0.34 (n=97)				1.53 ± 0.18 (n=53)	0.58 ± 0.2 (n=43)		
Weight (kg)	17.63 ± 0.48 (n=77)	51.74 ± 1.85 (n=97)	80.68 ± 4.58 (n=311)	84.80 ± 1.85 (n=110)	<0.001	17.27 ± 0.46 (n=53)	43.64 ± 2.3 (n=43)	75.40 ± 1.71 (n=88)	<0.001
Weight z-score	0.94 ± 0.14 (n=77)	1.14 ± 0.21 (n=97)				0.92 ± 0.13 (n=53)	0.66 ± 0.19 (n=43)		
BMI (kg/m^2)	16.56 ± 0.25 (n=76)	21.52 ± 0.5 (n=97)	29.11 ± 0.36 (n=311)	29.59 ± 0.6 (n=110)	<0.001	15.99 ± 0.15 (n=53)	19.21 ± 0.5 (n=43)	25.73 ± 0.47 (n=88)	<0.001
BMI z-score	0.26 ± 0.15 (n=76)	0.99 ± 0.13 (n=97)				-0.04 ± 0.1 (n=53)	0.56 ± 0.17 (n=43)		

	Severe Cohorts					Mild to Moderate Cohorts			
	Preschool children (SW)	**School aged children (SA)**	**Non-smoking adults (SAn)**	**Smokers and ex-smokers (SAs/ex)**	**p-value**	**Preschool children (SW)**	**School aged children (SA)**	**Non-smoking adults (SAn)**	**p-value**
n	77	97	311	110		54	43	88	
Asthma history									
Age at diagnosis (years)	1.74 ± 0.12 (n=73)	3.25 ± 0.27 (n=93)	23.99 ± 1.03 (n=302)	33.62 ± 1.82 (n=109)	<0.001	1.48 ± 0.13 (n=46)	3.78 ± 0.48 (n=41)	19.89 ± 1.83 (n=83)	<0.001
ICU admission ever	9/77 (11.7)	9/97 (19.6)	80/307 (26.1)	18/109 (16.5)	0.056	2/54 (3.7)	4/43 (9.3)	1/86 (1.2)	0.116
ICU admission in past year	6/77 (7.8)	5/97 (5.2)	13/310 (4.2)	4/110 (3.6)	0.802	2/54 (3.7)	1/43 (2.3)	0/88 (0.0)	0.217
Number of exacerbations in previous year	3.91 ± 0.36 (n=77)	3.87 ± 0.27 (n=97)	2.48 ± 0.13 (n=310)	2.55 ± 0.26 (n=110)	<0.001	1.83 ± 0.36 (n=54)	1.05 ± 0.21 (n=43)	0.38 ± 0.08 (n=88)	<0.001
Reported triggers for respiratory symptoms									
Respiratory infections	77/77 (100.0)	91/96 (94.8)	271/304 (89.1)	92/110 (83.6)	0.001	53/53 (100.0)	41/42 (97.6)	59/84 (70.2)	<0.001
Pets	14/60 (23.3)	62/92 (67.4)	139/287 (48.4)	34/105 (32.4)	<0.001	11/49 (22.4)	29/38 (76.3)	42/83 (50.6)	<0.001
Exercise	58/74 (78.4)	86/96 (89.6)	239/288 (83.0)	86/106 (81.1)	0.226	20/51 (39.2)	33/42 (19.0)	54/83 (65.1)	<0.001
Cold air	61/72 (84.7)	79/97 (81.4)	237/304 (78.0)	53/104 (51.0)	<0.001	24/53 (45.3)	24/42 (57.1)	50/84 (59.5)	0.248
Air pollutants	18/55 (32.7)	55/85 (64.7)	199/291 (68.4)	67/103 (65.0)	<0.001	5/47 (10.6)	17/37 (45.9)	39/80 (48.8)	<0.001

	Severe Cohorts					Mild to Moderate Cohorts			
	Preschool children (SW)	**School aged children (SA)**	**Non-smoking adults (SAn)**	**Smokers and ex-smokers (SAs/ex)**	**p-value**	**Preschool children (SW)**	**School aged children (SA)**	**Non-smoking adults (SAn)**	**p-value**
n	77	97	311	110		54	43	88	
Stress	24/63 (38.1)	55/92 (59.8)	168/295 (56.9)	56/105 (53.3)	0.034	5/51 (9.8)	18/43 (41.9)	25/85 (29.4)	0.002
Pollens	34/65 (52.3)	76/93 (81.7)	184/293 (62.8)	49/105 (46.7)	<0.001	9/49 (18.4)	31/42 (73.8)	48/82 (58.5)	<0.001
Spirometry									
FEV_1 % predicted	104.34 ± 3.21 (n=19)	88.68 ± 2.15 (n=96)	67.5 ± 1.26 (n=308)	67.21 ± 1.84 (n=110)	<0.001	99.23 ± 5.29 (n=10)	93.51 ± 2.47 (n=42)	89.48 ± 1.86 (n=87)	0.145
FEV_1 z-score	0.33 ± 0.24 (n=19)	-0.92 ± 0.18 (n=96)				-0.03 ± 0.4 (n=10)	-0.53 ± 0.2 (n=42)		
FVC % predicted	107.99 ± 3.5 (n=19)	102.15 ± 1.65 (n=96)	87.22 ± 1.12 (n=308)	89.72 ± 1.74 (n=110)	<0.001	103.54 ± 5.23 (n=10)	104.45 ± 2.02 (n=42)	104.45 ± 2.02 (n=87)	0.987
FVC z-score	0.55 ± 0.25 (n=19)	0.16 ± 0.14 (n=96)				0.25 ± 0.38 (n=10)	0.37 ± 0.17 (n=42)		
FEV_1/FVC ratio	0.91 ± 0.02 (n=19)	0.77 ± 0.01 (n=96)	0.64 ± 0.01 (n=308)	0.61 ± 0.01 (n=110)	<0.001	0.89 ± 0.02 (n=10)	0.80 ± 0.02 (n=42)	0.72 ± 0.01 (n=87)	<0.001

Data are presented as n/N (%) or mean ± SE (n). p-value were calculated using the Chi-squared test for categorical data or the one-way ANOVA test for continuous data.

5.2 Allergic Diseases

5.2.1 Allergic Diseases in the Severe Cohorts

The prevalence of diagnosed eczema was highest in school aged children at 79% compared to 57% in preschool children, 35% in non-smoking adults and 29% in adult smokers/ex-smokers. Of those with eczema, the proportion of participants whose eczema was currently active did not differ significantly between cohorts. The majority of preschool and school aged children with eczema were diagnosed in the first two years of life (83% and 63%, respectively), compared to only 23% of non-smoking adults and 11% of smokers/ex-smokers. 50% of smokers/ex-smokers and 43% of non-smokers developed eczema in adulthood. Allergic rhinitis was most prevalent in school aged children (64%) and least prevalent in preschool children (32%). A diagnosis of hay fever was also more common in school aged children (81% compared to 44% in the preschool children, 45% in non-smoking adults and 48% in smokers/ex-smokers). The prevalence of active hay fever was higher in both preschool and school aged children (89% and 92%, respectively) compared to non-smoking adults (70%) and smokers/ex-smokers (68%). In the severe paediatric cohorts most cases of allergic rhinitis and hay fever were diagnosed over the age of 2 years. Amongst adult smokers/ex-smokers with severe asthma, the majority of cases of allergic rhinitis and hay fever were diagnosed in adulthood (63% and 57%, respectively) (Table 16 and Figure 8).

5.2.2 Allergic Diseases in the Mild to Moderate Cohorts

The prevalence of diagnosed eczema was higher in preschool children and school aged children (59% and 65%, respectively) than non-smoking adults (29%). For hay fever, the highest prevalence was seen in school aged children (71%) followed by non-smoking adults (51%) and preschool children (22%). A similar pattern was seen for allergic rhinitis. The proportion of participants with active eczema or hay fever did not differ significantly between cohorts. Active allergic rhinitis was, however, more common amongst school aged children than preschool children and non-smoking adults (94% versus 77% and 79%, respectively). Similar to the severe cohorts, the majority of preschool and school aged children with eczema, developed this in the first two years of life (91% and 82%, respectively). Most children in the mild to moderate wheeze and mild to moderate asthma cohorts with allergic rhinitis or hay fever were, however, diagnosed in later childhood. Amongst non-smoking adults with mild to moderate asthma, similar numbers were diagnosed with hay fever or allergic rhinitis between the ages of 2 and 17 years and in adulthood (54% vs 46% for hay fever) (Table 17 and Figure 9).

Table 16 Prevalence of Allergic Diseases in the Severe UBIOPRED Cohorts

	Preschool children (SW)	**School aged children (SA)**	**Non-smoking adults (SAn)**	**Smokers and ex-smokers (SAs/ex)**	**p-value**
n	77	97	311	110	
Eczema					
Diagnosed	42/74 (56.7)	77/97 (79.4)	107/308 (34.7)	31/108 (28.7)	<0.001
Currently active	31/38 (81.6)	49/76 (64.4)	57/100 (57.0)	18/29 (62.1)	0.064
Age of onset (years)					
○ Less than 2	35/42 (83.3)	48/76 (63.2)	22/98 (22.5)	3/28 (10.7)	<0.001
○ 2 to 17	7/42 (16.7)	28/76 (36.8)	33/98 (33.7)	11/28 (39.3)	
○ 18 or more	NA	NA	43/98 (43.9)	14/28 (50.0)	
Allergic rhinitis					
Diagnosed	22/69 (31.9)	61/95 (64.2)	164/291 (56.4)	44/108 (40.7)	<0.001
Currently active	20/22 (90.9)	57/60 (95.0)	122/157 (77.7)	35/43 (81.4)	0.016
Age of onset (years)					
○ Less than 2	7/22 (31.8)	8/60 (13.3)	4/154 (2.6)	0/40 (0.0)	<0.001
○ 2 to 17	15/22 (68.2)	52/60 (88.7)	71/154 (46.1)	15/40 (37.5)	
○ 18 or more	NA	NA	79/154 (51.3)	25/40 (62.5)	
Hay fever					
Diagnosed	30/68 (44.1)	75/93 (80.7)	135/298 (45.3)	51/107 (47.7)	<0.001
Currently active	25/28 (89.3)	67/73 (91.8)	92/132 (69.7)	34/51 (66.7)	<0.001
Age of onset (years)					
○ Less than 2	11/30 (36.7)	7/74 (9.5)	7/127 (5.5)	2/49 (4.1)	**<0.001**
○ 2 to 17	19/30 (63.3)	67/74 (90.5)	74/127 (58.3)	19/49 (38.8)	
○ 18 or more	NA	NA	46/127 (36.2)	28/49 (57.1)	

Data are presented as n/N (%). p-values were calculated using the Chi-squared test.

The denominators and hence percentages for diagnosed eczema, allergic rhinitis and hay fever differ from those reported in the baseline UBIOPRED papers[142,143] due to a data processing error when preparing the baseline papers.

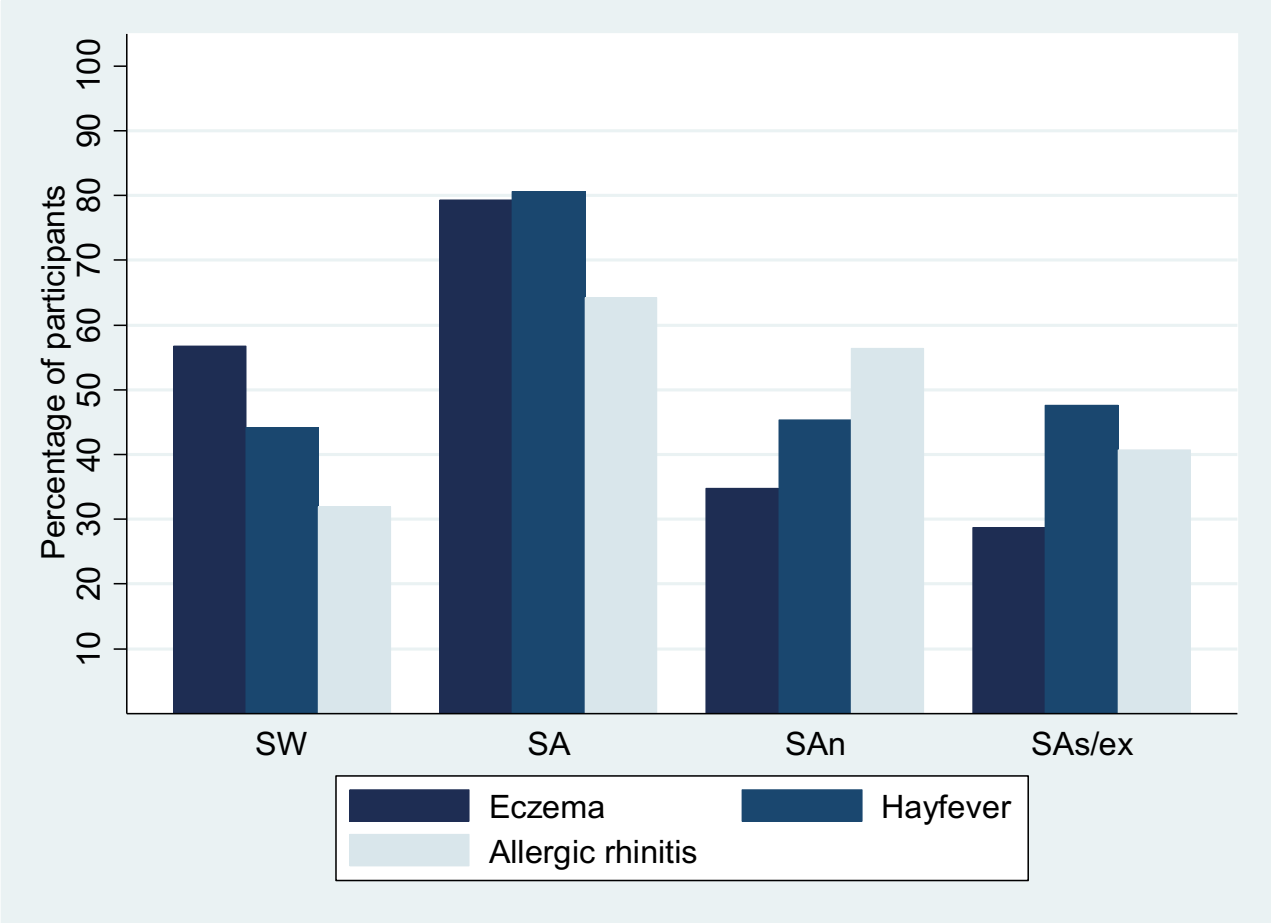

Figure 8 Bar Chart showing the Prevalence of Allergic Diseases in the Severe UBIOPRED Cohorts

Table 17 Prevalence of Allergic Diseases in the Mild to Moderate UBIOPRED Cohorts

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
Eczema n/N (%)				
Diagnosed	32/54 (59.3)	28/43 (65.1)	25/87 (28.7)	<0.001
Currently active	19/31 (61.3)	19/27 (70.4)	10/24 (41.7)	0.107
Age of onset (years)				
○ Less than 2	29/32 (90.6)	23/28 (82.1)	5/24 (20.8)	<0.001
○ 2 to 17	3/32 (9.4)	5/28 (17.9)	11/24 (45.8)	
○ 18 or more	NA	NA	8/24 (33.3)	
Allergic rhinitis n/N (%)				
Diagnosed	13/50 (26.0)	33/43 (76.7)	46/85 (54.1)	<0.001
Currently active	10/13 (76.9)	31/33 (93.9)	34/43 (79.1)	0.002
Age of onset (years)				
○ Less than 2	4/12 (33.3)	3/32 (9.4)	1/44 (2.3)	<0.001
○ 2 to 17	8/12 (66.6)	29/32 (90.6)	25/44 (56.8)	
○ 18 or more	NA	NA	18/44 (40.9)	
Hay fever n/N (%)				
Diagnosed	11/51 (21.6)	29/41 (70.7)	42/82 (51.2)	<0.001
Currently active	10/11 (90.9)	29/29 (100.0)	28/41 (68.3)	0.155
Age of onset (years)				
○ Less than 2	2/10 (20.0)	5/29 (17.2)	0/37 (0.0)	<0.001
○ 2 to 17	8/10 (80.0)	24/29 (82.8)	20/37 (54.0)	
○ 18 or more	NA	NA	17/37 (46.0)	

Data are presented as n/N (%). p-values were calculated using the Chi-squared test.

The denominators and hence percentages for diagnosed eczema, allergic rhinitis and hay fever differ from those reported in the baseline UBIOPRED papers[142,143] due to a data processing error when preparing the baseline papers.

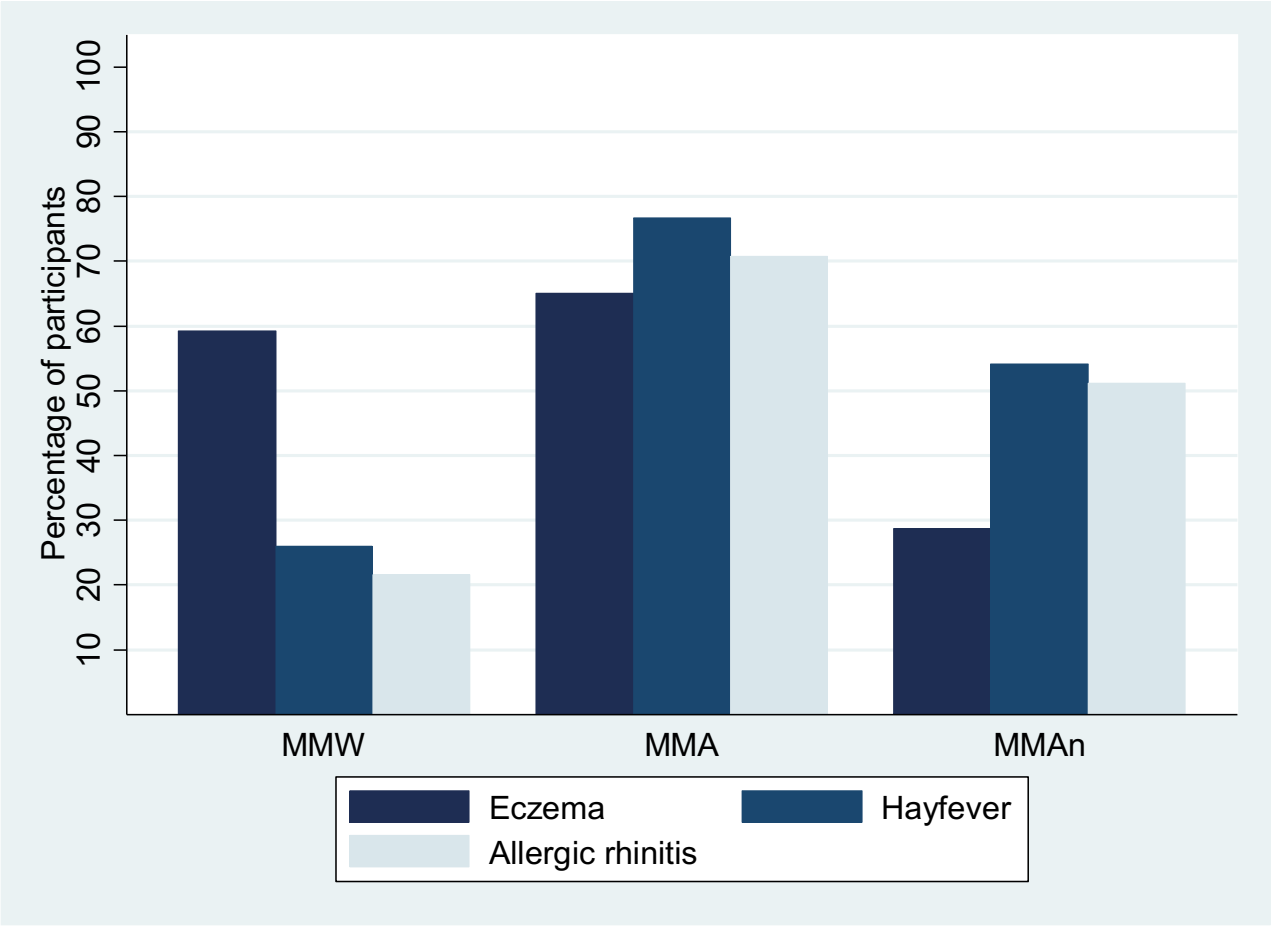

Figure 9 Bar Chart showing the Prevalence of Allergic Diseases in the Mild to Moderate UBIOPRED Cohorts

5.3 Food Allergy

5.3.1 Food Allergy in the Severe Cohorts

The prevalence of any possible food allergy ranged from 3.0% in adult smokers/ex-smokers to 31.1% in school aged children. The prevalence of possible food allergy was significantly higher in the severe school aged cohort than in all of the other severe cohorts but there were no significant differences between the other cohorts. When higher SPT and IgE cut-off values were used (to define highly likely allergy), this was also the case. The prevalence of highly likely allergy was 16.7% in school aged participants compared to 3.0% in non-smoking adults, 2.9% in preschool children and 2.0% in adult smokers/ex-smokers ($p<0.001$). The most common food allergy in all cohorts was peanut followed by tree nuts, egg and milk. For all of these foods, the prevalences of both possible allergy and highly likely allergy were significantly higher in the school aged cohort than the other cohorts. Higher prevalences of egg and milk allergy were also observed in preschool children compared to non-smoking adults (4.0 vs 0.7%, $p=0.025$ for possible milk allergy and 4.0 vs 0.3%, p0.005 for possible egg allergy) (Table 18, Table 19 and Figure 10). The skin prick test and specific IgE results of participants from the severe cohorts with food allergy can be found in tables 22-25.

5.3.2 Food Allergy in the Mild to Moderate Cohorts

Once again, the prevalence of food allergy was highest in school aged participants. The prevalence of any possible allergy was significantly higher in the school aged cohort compared to the non-smoking adult cohort (29.3 vs 6.1%, $p<0.001$) whilst the prevalence of highly likely food allergy was higher in the school aged cohort compared to both other cohorts. As for the severe cohorts, peanut and tree nut allergies were most common. For peanut, tree nuts and egg, the prevalence of allergy (possible and highly likely) was higher in school aged children compared to non-smoking adults. With the exception of highly likely tree nut allergy (0 vs 10%), there were however no significant differences between preschool and school aged children (Table 20, Table 21 and Figure 11). The skin prick test and specific IgE results of participants from the mild to moderate cohorts with food allergy can be found in tables 26-28.

Table 18 Prevalence of Food Allergy in the Severe UBIOPRED Cohorts

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
ANY FOOD ALLERGY					
Possible allergy	7/70 (10.0)	28/90 (31.1)	25/265 (9.4)	3/100 (3.0)	<0.001
Highly likely allergy	2/70 (2.9)	15/90 (16.7)	8/265 (3.0)	2/100 (2.0)	<0.001
Peanut allergy					
History of symptoms	5/76 (6.6)	21/97 (21.7)	33/308 (10.7)	4/109 (3.7)	<0.001
Doctor diagnosis	5/5 (100.0)	21/21 (100.0)	18/26 (69.2)	1/3 (33.3)	0.004
Age of onset (years)					
○ Less than 2	3/5 (60.0)	4/21 (19.1)	4/30 (13.3)	0/4 (0.0)	<0.001
○ 2 to 17	2/5 (40.0)	17/21 (81.0)	10/30 (33.3)	1/4 (25.0)	
○ 18 or more	NA	NA	16/30 (53.3)	3/4 (75.0)	
Evidence of sensitisation	6/19 (31.6)	24/34 (70.6)	11/31 (35.5)	1/4 (25.0)	0.008
Possible allergy	4/75 (5.3)	14/92 (15.2)	10/304 (3.3)	1/109 (0.9)	<0.001
Highly likely allergy	2/75 (2.7)	10/92 (10.9)	2/304 (0.7)	1/109 (0.9)	<0.001
Tree nut allergy					
History of symptoms	5/76 (6.6)	17/93 (18.3)	31/305 (10.2)	1/109 (0.9)	<0.001
Doctor diagnosis	4/4 (100.0)	16/16 (100.0)	16/23 (69.6)	-	0.026
Age of onset (years)					
○ Less than 2	3/5 (60.0)	4/17 (23.5)	3/27 (11.1)	0/1 (0.0)	0.002
○ 2 to 17	2/5 (40.0)	13/17 (76.5)	10/27 (37.0)	1/1 (100.0)	
○ 18 or more	NA	NA	14/27 (51.9)	0/0 (0.0)	
Evidence of sensitisation	4/4 (100.0)	16/17 (94.1)	20/40 (50.0)	1/5 (20.0)	0.001
Possible allergy	3/74 (4.1)	12/89 (13.5)	18/299 (6.0)	0/108 (0.0)	0.001
Highly likely allergy	1/74 (1.4)	8/89 (9.0)	6/299 (2.0)	0/108 (0.0)	<0.001

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
Milk allergy					
History of symptoms	6/76 (7.9)	17/96 (17.7)	14/304 (4.6)	6/110 (5.5)	<0.001
Doctor diagnosis	4/5 (80.0)	16/17 (94.1)	7/9 (77.8)	4/4 (100.0)	0.488
Age of onset (years)					
○ Less than 2	5/6 (83.3)	9/17 (52.9)	2/14 (14.3)	0/6 (0.0)	0.001
○ 2 to 17	1/6 (16.7)	8/17 (47.1)	5/14 (35.7)	2/6 (33.3)	
○ 18 or more	NA	NA	7/14 (50.0)	4/6 (66.6)	
Evidence of sensitisation	9/28 (32.1)	14/34 (41.2)	2/15 (13.3)	1/5 (20.0)	0.254
Possible allergy	3/76 (4.0)	8/94 (8.5)	2/303 (0.7)	1/109 (0.9)	<0.001
Highly likely allergy	0/76 (0.0)	2/94 (2.1)	0/303 (0.0)	1/110 (0.9)	0.070
Egg allergy					
History of symptoms	6/77 (7.8)	15/96 (15.6)	9/308 (2.9)	4/108 (3.7)	<0.001
Doctor diagnosis	6/6 (100.0)	14/14 (100.0)	5/6 (83.3)	3/3 (100.0)	0.265
Age of onset (years)					
○ Less than 2	5/6 (83.3)	8/15 (53.3)	1/8 (12.5)	0/4 (0.0)	0.012
○ 2 to 17	1/6 (16.7)	7/15 (46.7)	4/8 (50.0)	2/4 (50.0)	
○ 18 or more	NA	NA	3/8 (37.5)	2/4 (50.0)	
Evidence of sensitisation	4/20 (20.0)	15/30 (50.0)	1/9 (11.1)	1/3 (33.3)	0.063
Possible allergy	3/75 (4.0)	9.92 (9.8)	1/306 (0.3)	1/107 (0.9)	<0.001
Highly likely allergy	1/75 (1.3)	2/92 (2.2)	0/306 (0.0)	0/107 (0.0)	0.045

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
Fish allergy					
History of symptoms	2/77 (2.6)	2/97 (2.1)	11/307 (3.6)	1/110 (0.9)	0.493
Doctor diagnosis	2/2 (100.0)	2/2 (100.0)	4/7 (57.1)	1/1 (100.0)	0.414
Age of onset (years)					
○ Less than 2	1/2 (50.0)	0/2 (0.0)	1/10 (10.0)	0/1 (0.0)	0.072
○ 2 to 17	1/2 (50.0)	2/2 (100.0)	1/10 (10.0)	0/1 (0.0)	
○ 18 or more	NA	NA	8/10 (80.0)	1/1 (0.0)	
Evidence of sensitisation	0/0 (0.0)	1/1 (100.0)	1/10 (10.0)	0/1 (0.0)	0.063
Possible allergy	0/75 (0.0)	1/96 (1.0)	1/305 (0.3)	0/110 (0.0)	0.786
Highly likely allergy	0/75 (0.0)	0/96 (0.0)	1/305 (0.3)	0/110 (0.0)	0.820
Shell fish allergy					
History of symptoms	0/74 (0.0)	6/93 (6.5)	15/303 (5.0)	5/109 (4.6)	0.215
Doctor diagnosis	NA	6/6 (100.0)	7/12 (58.3)	2/5 (40.0)	0.088
Age of onset (years)					
○ Less than 2	NA	0/0 (0.0)	1/13 (7.7)	0/5 (0.0)	0.036
○ 2 to 17	NA	6/6 (100.0)	3/13 (23.1)	2/5 (40.0)	
○ 18 or more	NA	NA	9/13 (69.2)	3/5 (60.0)	
Evidence of sensitisation	0/0 (0.0)	1/3 (33.3)	3/16 (18.8)	0/4 (0.0)	0.498
Possible allergy	0/74 (0.0)	1/90 (1.1)	3/300 (1.0)	0/108 (0.0)	0.595
Highly likely allergy	0/74 (0.0)	1/90 (1.1)	0/300 (0.0)	0/108 (0.0)	0.147

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
OTHER FOOD ALLERGIES *					
Possible allergy	2/71 (2.8)	9/90 (10.0)	2/278 (0.7)	1/104 (1.0)	<0.001
Highly likely allergy	0/71 (0.0)	3/90 (3.3)	1/278 (0.4)	0/104 (0.0)	0.017

Data are presented as n/N (%). p-values were calculated using the Chi-squared test.

Symptoms of food allergy refers to symptoms of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with food.

Sensitisation is defined as a positive skin prick test (≥3mm wheal) or a positive specific IgE (≥ 0.35 kU/l).

Possible food allergy is defined as symptoms of food allergy plus evidence of sensitisation.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm skin prick test wheal or a specific IgE level ≥ 10.0 kU/l.

*Foods include wheat, soy, kiwi, sesame, celery, thyme and chocolate.

Table 19 Pairwise Comparisons of Food Allergy Prevalence in the Severe UBIOPRED Cohorts

	SW vs SA	SW vs SAn	SW vs SAs/ex	SA vs SAn	SA vs SAs/ex	SAn vs SAs/ex
Possible food allergy						
Any food	10.0 vs 31.1 (0.001)	10.0 vs 9.4 (0.886)	10.0 vs 3.0 (0.056)	31.1 vs 9.4 (<0.001)	31.1 vs 3.0 (<0.001)	9.4 vs 3.0 (0.039)
Peanut	5.3 vs 15.2 (0.040)	5.3 vs 3.3 (0.401)	5.3 vs 0.9 (0.070)	15.2 vs 3.3 (<0.001)	15.2 vs 0.9 (<0.001)	3.3 vs 0.9 (0.187)
Tree nuts	4.1 vs 13.5 (0.038)	4.1 vs 6.1 (0.511)	4.1 vs 0.0 (0.035)	13.5 vs 6.0 (0.021)	13.5 vs 0.0 (<0.001)	6.0 vs 0.0 (0.009)
Milk	4.0 vs 8.5 (0.229)	4.0 vs 0.7 (0.025)	4.0 vs 0.9 (0.163)	8.5 vs 0.7 (<0.001)	8.5 vs 0.9 (0.009)	0.7 vs 0.9 (0.786)
Egg	4.0 vs 9.8 (0.150)	4.0 vs 0.3 (0.005)	4.0 vs 0.9 (0.165)	9.8 vs 0.3 (<0.001)	9.8 vs 0.9 (0.004)	0.3 vs 0.9 (0.436)
Fish	0.0 vs 1.0 (0.375)	0.0 vs 0.3 (0.620)	0.0 vs 0.0	1.0 vs 0.3 (0.387)	1.0 vs 0.0 (0.283)	0.3 vs 0.0 (0.548)
Shell fish	0.0 vs 1.1 (0.363)	0.0 vs 1.0 (0.388)	0.0 vs 0.0	1.1 vs 1.0 (0.927)	1.1 vs 0.0 (0.272)	1.0 vs 0.0 (0.297)
Highly likely food allergy						
Any food	2.9 vs 16.7 (0.005)	2.9 vs 3.0 (0.944)	2.9 vs 2.0 (0.717)	16.7 vs 3.0 (<0.001)	16.7 vs 2.0 (<0.001)	3.0 vs 2.0 (0.595)
Peanut	2.7 vs 10.9 (0.041)	2.7 vs 0.7 (0.127)	2.7 vs 0.9 (0.357)	10.9 vs 0.7 (<0.001)	10.9 vs 0.9 (0.002)	0.7 vs 0.9 (0.784)
Tree nuts	1.4 vs 9.0 (0.034)	1.4 vs 2.0 (0.710)	1.4 vs 0.0 (0.226)	9.0 vs 2.0 (0.002)	9.0 vs 0.0 (0.001)	2.0 vs 0.0 (0.138)
Milk	0.0 vs 2.1 (0.201)	0.0 vs 0.0	0.0 vs 0.9 (0.402)	2.1 vs 0.0 (0.011)	2.1 vs 0.9 (0.476)	0.0 vs 0.9 (0.095)
Egg	1.3 vs 2.2 (0.684)	1.3 vs 0.0 (0.043)	1.3 vs 0.0 (0.231)	2.2 vs 0.0 (0.010)	2.2 vs 0.0 (0.125)	0.0 vs 0.0
Fish	0.0 vs 0.0	0.0 vs 0.3 (0.620)	0.0 vs 0.0	0.0 vs 0.3 (0.574)	0.0 vs 0.0	0.3 vs 0.0 (0.548)
Shell fish	0.0 vs 1.1 (0.363)	0.0 vs 0.0	0.0 vs 0.0	1.1 vs 0.0 (0.068)	1.1 vs 0.0 (0.272)	0.0 vs 0.0

Values represent percentages (p-value).

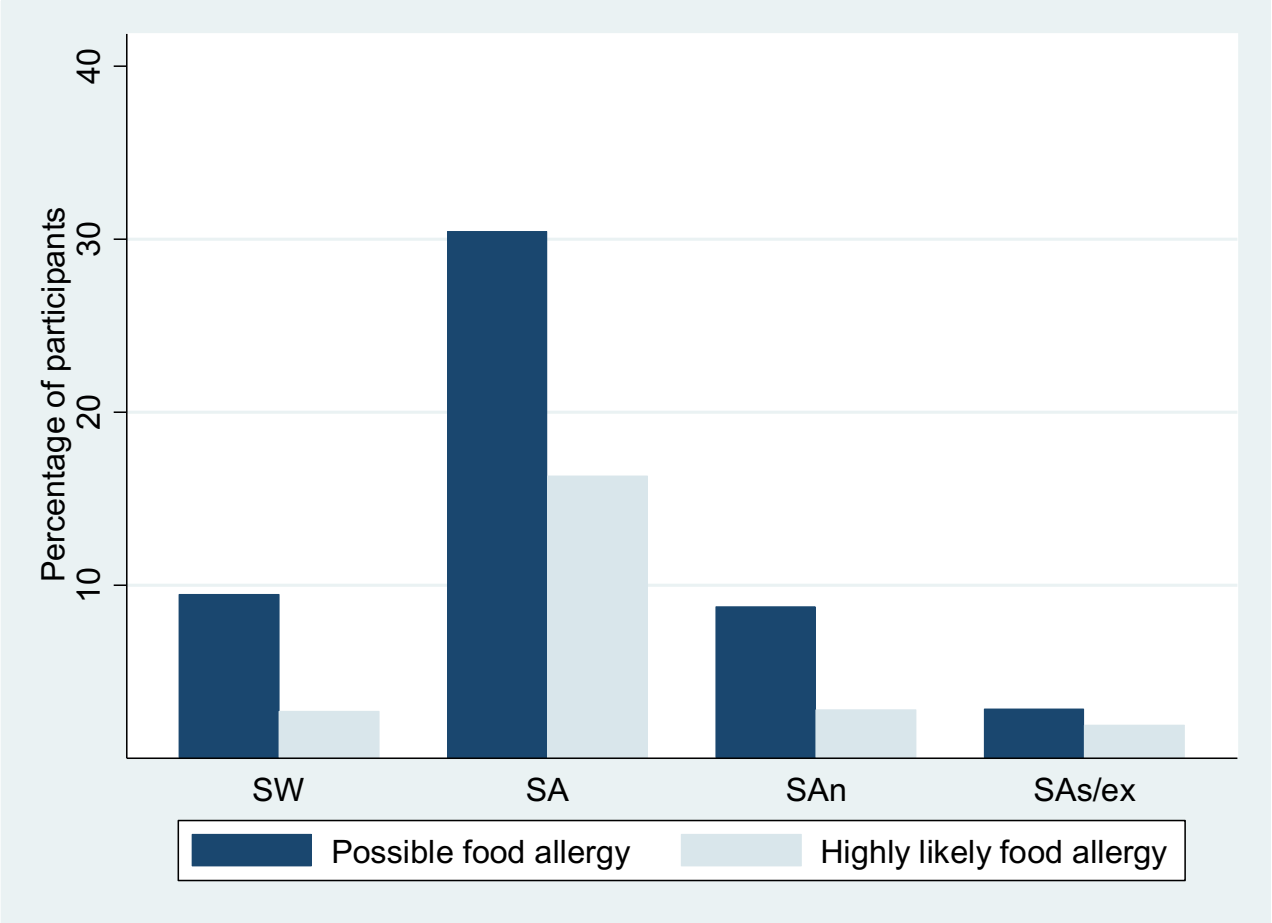

Figure 10 Bar Chart showing the Prevalence of Food Allergy in the Severe UBIOPRED Cohorts

Table 20 Prevalence of Food Allergy in the Mild to Moderate UBIOPRED Cohorts

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
ANY FOOD ALLERGY				
Possible food allergy	8/48 (16.7)	12/41 (29.3)	5/82 (6.1)	0.003
Highly likely food allergy	2/48 (4.2)	8/41 (19.5)	1/82 (1.2)	<0.001
Peanut allergy				
History of symptoms	8/54 (14.8)	11/43 (25.6)	5/88 (5.7)	
Doctor diagnosis	7/8 (87.5)	10/11 (90.9)	4/5 (80.0)	0.829
Age of onset (years)				
○ Less than 2	4/8 (50.0)	2/10 (20.0)	0/5 (0.0)	0.026
○ 2 to 17	4/8 (50.0)	8/10 (80.0)	3/5 (60.0)	
○ 18 or more	NA	NA		
Evidence of sensitisation	9/22 (40.9)	12/15 (80.0)	1/5 (20.0)	0.020
Possible allergy	6/52 (11.5)	8/41 (19.5)	1/88 (1.1)	0.001
Highly likely allergy	2/52 (3.9)	6/41 (14.6)	1/88 (1.1)	0.004
Tree nut allergy				
History of symptoms	5/51 (9.8)	7/42 (16.7)	7/88 (8.0)	0.311
Doctor diagnosis	5/5 (100.0)	7/7 (100.0)	5/7 (71.4)	0.147
Age of onset (years)				
○ Less than 2	2/5 (40.0)	3/6 (50.0)	2/7 (28.6)	0.448
○ 2 to 17	3/5 (60.0)	3/6 (50.0)	3/7 (42.9)	
○ 18 or more	NA	NA	2/7 (28.6)	
Evidence of sensitisation	4/4 (100.0)	8/8 (100.0)	7/10 (0.7)	0.124
Possible allergy	2/48 (4.2)	5/40 (12.5)	5/87 (5.8)	0.258
Highly likely allergy	0/48 (0.0)	4/40 (10.0)	1/87 (1.2)	0.008

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
Milk allergy				
History of symptoms	5/53 (9.4)	4/43 (7.0)	1/88 (1.1)	0.067
Doctor diagnosis	4/4 (100.0)	3/3 (100.0)	1/1 (100.0)	-
Age of onset (years)				
○ Less than 2	4/5 (80.0)	2/3 (66.7)	0/1 (0.0)	0.155
○ 2 to 17	1/5 (20.0)	1/3 (33.3)	1/1 (100.0)	
○ 18 or more	NA	NA		
Evidence of sensitisation	8/24 (33.3)	7/17 (41.2)	0/1 (0.0)	0.658
Possible allergy	2/51 (3.9)	2/42 (4.8)	0/88 (0.0)	0.139
Highly likely allergy	1/51 (2.0)	1/42 (2.4)	0/88 (0.0)	0.377
Egg allergy				
History of symptoms	5/53 (9.4)	5/43 (11.6)	1/88 (1.1)	0.027
Doctor diagnosis	5/5 (100.0)	5/5 (100.0)	1/1 (100.0)	-
Age of onset (years)				
○ Less than 2	4/5 (80.0)	4/4 (100.0)	1/1 (0.0)	0.574
○ 2 to 17	1/5 (20.0)	0/4 (0.0)	0/4 (0.0)	
○ 18 or more	NA	NA		
Evidence of sensitisation	5/20 (25.0)	5/11 (45.5)	0/1 (0.0)	0.396
Possible allergy	2/51 (3.9)	4/42 (9.5)	0/88 (0.0)	0.017
Highly likely allergy	0/51 (0.0)	2/42 (4.8)	0/88 (0.0)	0.035
History of symptoms	3/54 (5.6)	1/42 (2.4)	2/88 (2.3)	0.528

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
Fish allergy				
Doctor diagnosis	2/3 (66.6)	1/1 (100.0)	1/1 (100.0)	0.659
Age of onset (years)				
○ Less than 2	2/3 (66.7)	1/1 (100.0)	0/2 (0.0)	0.384
○ 2 to 17	1/3 (33.3)	0/1 (0.0)	1/2 (50.0)	
○ 18 or more	NA	NA	1/2 (50.0)	
Evidence of sensitisation	1/1 (100.0)	1/1 (100.0)	1/2 (50.0)	0.513
Possible allergy	1/52 (1.9)	1/42 (2.4)	1/88 (1.1)	0.858
Highly likely allergy	0/52 (0.0)	1/42 (2.4)	0/88 (0.0)	0.187
Shell fish allergy				
History of symptoms	1/52 (1.9)	1/39 (2.6)	1/86 (1.2)	0.223
Doctor diagnosis	1/1 (100.0)	1/1 (100.0)	1/1 (100.0)	-
Age of onset				
○ Less than 2	0/1 (0.0)	1/1 (100.0)	0/1 (0.0)	-
○ 2 to 17	1/1 (100.0)	0/1 (0.0)	1/1 (100.0)	
○ 18 or more	NA	NA		
Evidence of sensitisation	0/0 (0.0)	0/2 (0.0)	1/1 (100.0)	0.083
Possible allergy	0/51 (0.0)	0/38 (0.0)	1/86 (1.2)	0.594
Highly likely allergy	0/51 (0.0)	0/38 (0.0)	0/86 (0.0)	-

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
OTHER FOOD ALLERGIES *				
Possible allergy	2/52 (3.9)	4/41 (9.8)	0/82 (0.0)	0.019
Highly likely allergy	1/52 (1.9)	0/41 (0.0)	0/82 (0.0)	0.304

Data are presented as n/N (%). p-values were calculated using the Chi-squared test.

Symptoms of food allergy refers to symptoms of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with food.

Sensitisation is defined as a positive skin prick test (≥3mm wheal) or a positive specific IgE (≥ 0.35 kU/l).

Possible food allergy is defined as symptoms of food allergy plus evidence of sensitisation.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm skin prick test wheal or a specific IgE level ≥ 10.0 kU/l.

*****Foods include wheat, soy, coconut, tomato, lime and sesame.

Table 21 Pairwise Comparisons of Food Allergy Prevalence in the Mild to Moderate UBIOPRED Cohorts

	MMW vs MMA	MMW vs MMAn	MMA vs MMAn
Possible food allergy			
Any food	16.7 vs 29.3 (0.156)	16.7 vs 6.1 (0.053)	29.3 vs 6.1 (<0.001)
Peanut	11.5 vs 19.5 (0.286)	11.5 vs 1.1 (0.006)	19.5 vs 1.1 (<0.001)
Tree nuts	4.2 vs 12.5 (0.150)	4.2 vs 5.8 (0.692)	12.5 vs 5.8 (0.189)
Milk	3.9 vs 4.8 (0.842)	3.9 vs 0.0 (0.061)	4.8 vs 0.0 (0.039)
Egg	3.9 vs 9.5 (0.274)	3.9 vs 0.0 (0.061)	9.5 vs 0.0 (0.003)
Fish	1.9 vs 1.1 (0.705)	2.4 vs 1.1 (0.590)	0.0 vs 0.0
Shell fish	0.0 vs 0.0	0.0 vs 1.2 (0.440)	0.0 vs 1.2 (0.505)
Highly likely food allergy			
Any food	4.2 vs 19.5 (0.022)	4.2 vs 1.2 (0.280)	19.5 vs 1.2 (<0.001)
Peanut	3.9 vs 14.6 (0.065)	3.9 vs 1.1 (0.285)	14.6 vs 1.1 (0.002)
Tree nuts	0.0 vs 10.0 (0.025)	0.0 vs 1.2 (0.453)	10.0 vs 1.2 (0.018)
Milk	2.0 vs 2.4 (0.889)	2.0 vs 0.0 (0.187)	2.4 vs 0.0 (0.146)
Egg	0.0 vs 4.8 (0.115)	0.0 vs 0.0	4.8 vs 0.0 (0.039)
Fish	0.0 vs 2.4 (0.263)	0.0 vs 0.0	2.4 vs 0.0 (0.146)
Shell fish	0.0 vs 0.0	0.0 vs 0.0	0.0 vs 0.0

Values represent percentages (p-value).

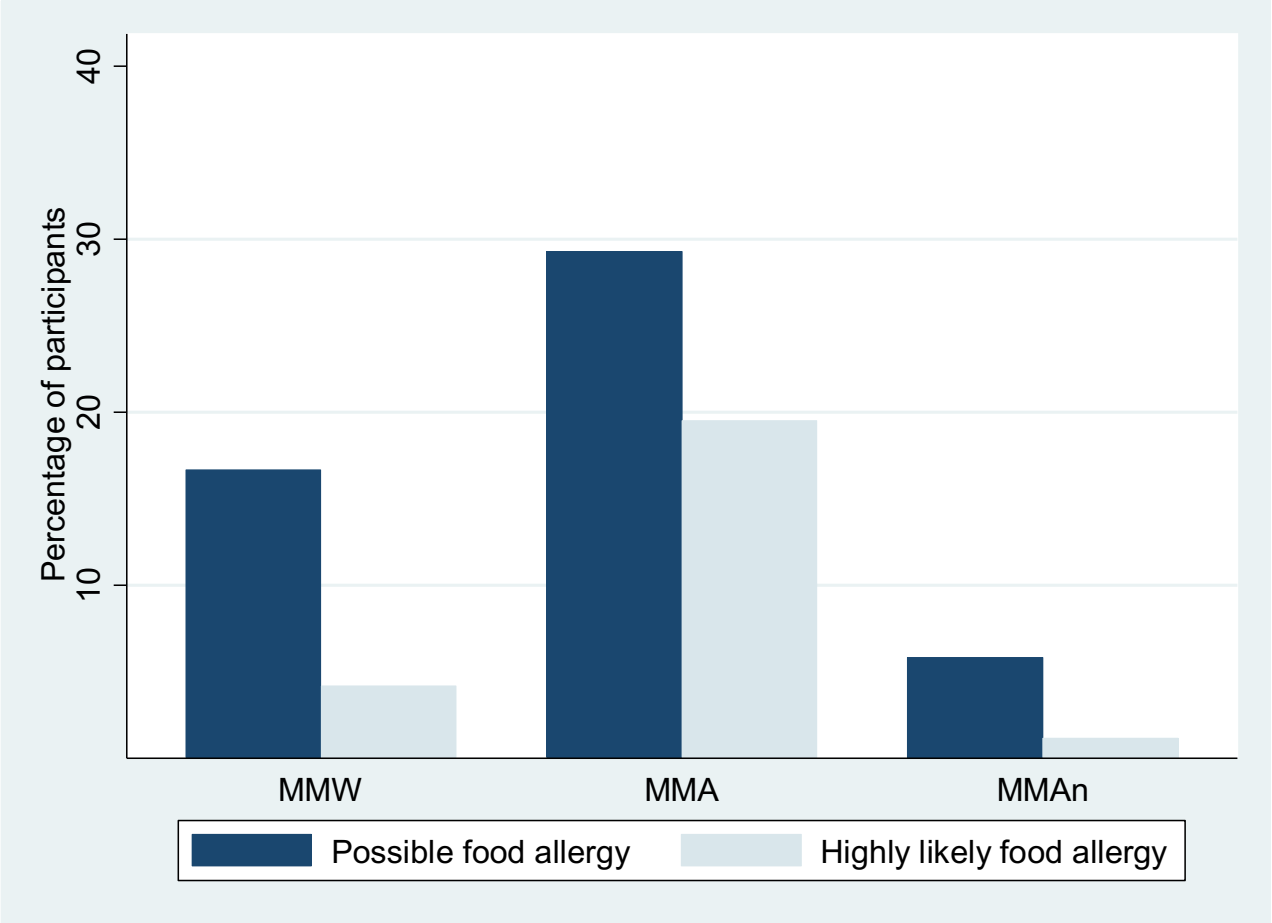

Figure 11 Bar Chart Showing the Prevalence of Food Allergy in the Mild to Moderate UBIOPRED Cohorts

Table 22 Details of Food Allergy Diagnostics in the Severe Preschool Wheeze Cohort

Preschool children with severe wheeze (SW)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
P_077	Peanut	NA	19.0	Highly likely allergy
	Milk	NA	1.1	Possible allergy
	Egg	NA	100	Highly likely allergy
P_090	Thyme	0	0.35	Possible allergy
P_112	Egg	NA	0.52	Possible allergy
P_139	Peanut	NA	25.8	Highly likely allergy
	Tree nuts	NA	55.9	Highly likely allergy
	Milk	NA	8.27	Possible allergy
	Egg	NA	8.11	Possible allergy
P_187	Milk	2	0.35	Possible allergy
	Soy	1	0.35	Possible allergy
P_198	Peanut	3	7.9	Possible allergy
	Tree nuts	NA	5.99	Possible allergy
P_268	Peanut	NA	1.2	Possible allergy
	Tree nuts	NA	3.13	Possible allergy

NA= Not available.

Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or specific IgE level ≥ 0.35kU/l.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or specific IgE level ≥ 10.0 kU/l.

Table 23 Details of Food Allergy Diagnostics in the Severe School Aged Asthma Cohort

School aged children with severe asthma (SA)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
P_006	Soy	NA	1.84	Possible allergy
P_007	Tree nuts	6 (hazelnut)	49.7	Highly likely allergy
P_016	Peanut	NA	2.59	Possible allergy
	Tree nuts	NA	47.3	Highly likely allergy
	Sesame	NA	32.7	Highly likely allergy
P_021	Peanut	0	1.12	Possible allergy
	Tree nuts	NA	0.37	Possible allergy
P_029	Shellfish	NA	16.1	Highly likely allergy
P_045	Milk	NA	7.8	Possible allergy
	Egg	NA	0.4	Possible allergy
P_055	Peanut	NA	25.3	Highly likely allergy
	Tree nuts	NA	65.5	Highly likely allergy
	Egg	NA	4.61	Possible allergy
	Soy	NA	0.92	Possible allergy
P_079	Soy	NA	1.47	Possible allergy
P_086	Peanut	10	41.7	Highly likely allergy
	Celery	NA	65	Highly likely allergy
P_101	Peanut	NA	1.84	Possible allergy
P_109	Tree nuts	9 (hazelnut)	NA	Highly likely allergy
P_114	Egg	NA	1.7	Possible allergy
P_126	Egg	NA	1.01	Possible allergy
P_131	Peanut	NA	41.1	Highly likely allergy
	Milk	NA	100	Highly likely allergy
	Egg	NA	57.1	Highly likely allergy
	Wheat	NA	71.4	Highly likely allergy
P_152	Peanut	NA	77.2	Highly likely allergy
	Tree nuts	6 (hazelnut)	NA	Highly likely allergy
	Fish	NA	4.2	Possible allergy
	Milk	NA	28	Highly likely allergy
P_154	Milk	NA	0.41	Possible allergy
P_166	Peanut	NA	3.52	Possible allergy
	Tree nuts	NA	18.6	Highly likely allergy

School aged children with severe asthma (SA)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
	Egg	NA	0.65	Possible allergy
	Sesame	NA	1.45	Possible allergy
P_174	Peanut	NA	21.0	Highly likely allergy
	Tree nuts	NA	22.8	Highly likely allergy
P_176	Peanut	5	100	Highly likely allergy
P_180	Peanut	6	0.64	Highly likely allergy
	Tree nuts	NA	3.32	Possible allergy
P_191	Milk	NA	3.0	Possible allergy
	Egg	NA	1.5	Possible allergy
P_196	Milk	4	NA	Possible allergy
P_200	Egg	NA	4	Possible allergy
P_214	Milk	NA	5.3	Possible allergy
	Chocolate	NA	0.4	Possible allergy
P_221	Peanut	NA	81.5	Highly likely allergy
	Tree nuts	NA	14.7	Highly likely allergy
	Kiwi	NA	6.31	Possible allergy
P_229	Peanut	5	100	Highly likely allergy
	Milk	NA	0.69	Possible allergy
	Egg	NA	100	Highly likely allergy
P_230	Tree nuts	NA	4.32	Possible allergy
P_259	Peanut	9	11.9	Highly likely allergy
	Tree nuts	NA	8.51	Possible allergy

NA= Not available.

Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or specific IgE level ≥ 0.35kU/l.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or specific IgE level ≥ 10.0 kU/l.

Table 24 Details of Food Allergy Diagnostics in Non-smoking Adults with Severe Asthma

Non-smoking adults with severe asthma (SAn)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
A_045	Milk	NA	7.48	Possible food allergy
	Wheat	NA	25	Highly likely allergy
A_064	Tree nuts	NA	2.04	Possible allergy
A_072	Tree nuts	NA	8.04	Possible allergy
A_098	Peanut	NA	1.74	Possible allergy
	Tree nuts	NA	35.7	Highly likely allergy
A_107	Tree nuts	NA	5.37	Possible allergy
	Kiwi	NA	0.5	Possible allergy
A_110	Peanut	NA	0.95	Possible allergy
	Tree nuts	NA	17.1	Highly likely allergy
	Fish	NA	11.2	Highly likely allergy
	Shellfish	NA	7.59	Possible allergy
A_149	Tree nuts	NA	15.1	Highly likely allergy
A_157	Egg	NA	0.37	Possible allergy
A_191	Tree nuts	NA	0.63	Possible allergy
A_192	Tree nuts	NA	0.41	Possible allergy
A_214	Peanut	NA	1.22	Possible allergy
	Tree nuts	NA	9.09	Possible allergy
	Shellfish	NA	3.65	Possible allergy
A_275	Peanut	NA	1.57	Possible allergy
	Tree nuts	NA	0.46	Possible allergy
A_317	Tree nuts	NA	0.69	Possible allergy
A_403	Peanut	NA	100	Highly likely allergy
	Tree nuts	NA	100	Highly likely allergy
A_466	Peanut	NA	31.4	Highly likely allergy
A_469	Peanut	NA	0.44	Possible allergy
	Tree nuts	NA	25.8	Highly likely allergy
A_475	Peanut	NA	1.61	Possible allergy

Non-smoking adults with severe asthma (SAn)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
A_482	Milk	NA	0.47	Possible allergy
	Egg	NA	4.18	Possible allergy
A_519	Tree nuts	NA	14.0	Highly likely allergy
A_528	Tree nuts	NA	1.93	Possible allergy
A_543	Tree nuts	NA	0.6	Possible allergy
550	Tree nuts	NA	2.68	Possible allergy
A_643	Peanut	NA	1.4	Possible allergy
A_648	Shellfish	NA	1.48	Possible allergy
A_698	Peanut	NA	0.93	Possible allergy
	Tree nuts	NA	2.3	Possible allergy

NA= Not available.

Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or specific IgE level ≥ 0.35kU/l.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or specific IgE level ≥ 10.0 kU/l.

Table 25 Details of Food Allergy Diagnostics in Smokers/Ex-smokers with Severe Asthma

Smokers/ex-smokers with severe asthma (SAs/ex)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
A_435	Peanut	NA	10.3	Highly likely allergy
A_488	Milk	NA	10.1	Highly likely allergy
A_583	Wheat	NA	0.66	Possible allergy

Table 26 Details of Food Allergy Diagnostics in the Mild to Moderate Preschool Wheeze Cohort

Preschool children with mild/moderate wheeze (MMW)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
P_062	Tree nuts	NA	1.61	Possible allergy
	Fish	NA	4.21	Possible allergy
	Milk	0	0.63	Possible allergy
P_115	Peanut	NA	7.58	Possible allergy
P_119	Lime	NA	0.35	Possible allergy
P_179	Peanut	NA	0.48	Possible allergy
P_213	Peanut	NA	10.4	Highly likely allergy
	Egg	NA	3.7	Possible allergy
P_272	Peanut	NA	70.3	Highly likely allergy
	Milk	NA	36.7	Highly likely allergy
	Egg	NA	9.83	Possible allergy
	Wheat	NA	10.2	Highly likely allergy
P_280	Peanut	NA	0.44	Possible allergy
	Tree nuts	NA	0.52	Possible allergy
P_285	Peanut	NA	1.56	Possible allergy

NA= Not available.
Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or a specific IgE level ≥ 0.35kU/l.
Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or a specific IgE level ≥ 10.0 kU/l.

Table 27 Details of Food Allergy Diagnostics in the Mild to Moderate School Aged Asthma Cohort

School aged children with mild to moderate asthma (MMA)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
P_015	Peanut	NA	14.9	Highly likely allergy
	Tree nuts	NA	98.6	Highly likely allergy
	Coconut	NA	4.85	Possible allergy
P_027	Peanut	3	6.63	Possible allergy
	Tree nuts	3 (hazelnut)	1.53	Possible allergy
	Soy	NA	0.47	Possible allergy
P_030	Tomato	NA	0.4	Possible allergy
P_072	Peanut	NA	11.1	Highly likely allergy
P_103	Milk	NA	100	Highly likely allergy
	Egg	4	100	Highly likely allergy
P_140	Peanut	8	43.6	Highly likely allergy
	Tree nuts	4 (hazelnut)	67.2	Highly likely allergy
	Sesame	4	NA	Possible allergy
P_185	Peanut	NA	11.0	Highly likely allergy
	Tree nuts	NA	10.4	Highly likely allergy
	Egg	NA	6.26	Possible allergy
P_195	Peanut	NA	1.77	Possible allergy
	Fish	NA	18.2	Highly likely allergy
P_202	Peanut	11	12.2	Highly likely allergy
	Egg	4	1.62	Possible allergy
P_263	Milk	NA	0.52	Possible allergy
P_292	Egg	NA	0.61	Possible allergy
P_295	Peanut	NA	100	Highly likely allergy
	Tree nuts	NA	100	Highly likely allergy

NA= Not available.

Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or a specific IgE level ≥ 0.35kU/l.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or a specific IgE level ≥ 10.0 kU/l.

Table 28 Details of Food Allergy Diagnostics in Non-smoking Adults with Mild to Moderate Asthma

Non-smoking adults with mild/moderate asthma (MMAn)				
Subject ID	**Food**	**SPT wheal size (mm)**	**Specific IgE (kU/l)**	**Outcome**
A_023	Tree nuts	NA	0.73	Possible allergy
A_156	Tree nuts	3.5 (hazelnut)	NA	Possible allergy
A_320	Tree nuts	NA	1.73	Possible allergy
A_362	Peanut	NA	12.6	Highly likely allergy
	Tree nuts	NA	33.1	Highly likely allergy
A_410	Tree nuts	NA	2.35	Possible allergy
	Fish	NA	0.99	Possible allergy
	Shell fish	NA	0.42	Possible allergy

NA= Not available.

Possible food allergy is defined as symptoms of food allergy plus a ≥3mm SPT wheal or a specific IgE level ≥ 0.35kU/l.

Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm SPT wheal or a specific IgE level ≥ 10.0 kU/l.

5.4 Atopy

5.4.1 Atopy in the Severe Cohorts

The prevalence of atopy was significantly lower in the severe preschool wheeze cohort than all other severe cohorts (42.9% vs 88.8% in school aged children, 80.5% in non-smoking adults and 66.7% in smokers/ex-smokers). It was similar in school aged children and non-smoking adults (88.8 vs 80.5, p=0.073). The prevalence of atopy in these cohorts was, however, higher than in the cohort consisting of adult smokers/ex-smokers with severe asthma. The median number of allergens to which participants in the severe school aged asthma cohort were sensitised was 4, compared to 0 in the severe preschool wheeze cohort (p<0.001) and 1 in each of the other severe cohorts (p<0.001). School aged children were most commonly sensitised to cat (66.7%), followed by house dust mite (64.4%) and grass pollen (62.9%). Amongst the other cohorts, house dust mite was the most common allergen to which participants were sensitised (Table 29, Table 30, Figure 12 and Figure 13).

5.4.2 Atopy in the Mild to Moderate cohorts

The prevalence of atopy was the same in school aged children and non-smoking adults with mild to moderate asthma (89.7%). This compares to 41.7% in preschool children with mild to moderate wheeze (p<0.001). On average, school aged children with mild to moderate asthma were sensitised to 4 allergens compared to 0 for preschool children with mild to moderate wheeze (p<0.001) and 3 for non-smoking adults with mild to moderate asthma (p=0.084). Similar to the severe school aged asthma cohort, the three most common allergens to which school aged children with mild to moderate asthma were sensitised were grass pollen (74.4%), house dust mite (70.0%) and cat (61.5%). These were also the three most common allergens to which non-smoking adults with mild to moderate asthma were sensitised. Preschool children with mild to moderate wheeze were most commonly sensitised to cat (28.9%), dog (28.9%) and grass pollen (20.0%) (Table 31, Table 32, Figure 14 and Figure 15).

Table 29 Prevalence of Allergic Sensitisation in the Severe UBIOPRED Cohorts

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
Positive skin prick test (≥3mm)					
Tree pollen	7/61 (11.5)	33/79 (41.8)	73/150 (48.7)	23/46 (50.0)	<0.001
Grass pollen	7/64 (10.9)	47/83 (56.6)	90/162 (55.6)	23/48 (47.9)	<0.001
Dog	6/64 (9.4)	43/83 (51.8)	58/122 (47.5)	16/43 (37.2)	<0.001
Cat	10/65 (15.4)	45/83 (54.2)	88/163 (54.0)	21/45 (46.7)	<0.001
House dust mite	16/63 (25.4)	42/83 (50.6)	102/176 (58.0)	26/51 (51.0)	<0.001
Mould	2/58 (3.5)	19/81 (23.5)	44/143 (30.8)	17/44 (38.6)	<0.001
Positive specific IgE (≥0.35 kU/l)					
Tree pollen	2/20 (10.0)	22/34 (64.7)	33/99 (33.3)	10/48 (20.8)	<0.001
Grass pollen	3/22 (13.6)	25/32 (78.1)	64/164 (39.0)	18/62 (29.0)	<0.001
Dog	3/21 (14.3)	20/27 (74.1)	57/166 (34.3)	12/54 (22.2)	<0.001
Cat	2/21 (9.5)	22/28 (78.6)	57/165 (34.6)	7/53 (13.2)	<0.001
House dust mite	9/20 (45.0)	27/34 (79.4)	79/168 (47.0)	21/60 (35.0)	<0.001
Mould	3/24 (12.5)	23/34 (67.7)	34/141 (24.1)	14/52 (26.9)	<0.001
Sensitisation *					
Tree pollen	9/65 (13.9)	45/84 (53.6)	90/208 (43.3)	28/82 (34.2)	<0.001
Grass pollen	9/69 (13.0)	56/89 (62.9)	119/260 (45.8)	32/92 (34.8)	<0.001
Dog	8/67 (11.9)	55/88 (62.5)	97/250 (38.8)	26/88 (30.0)	<0.001
Cat	11/69 (15.9)	58/87 (66.7)	114/259 (44.0)	25/86 (29.1)	<0.001
House dust mite	21/67 (31.3)	58/90 (64.4)	142/273 (52.0)	41/96 (42.7)	<0.001
Mould	5/63 (7.9)	37/87 (42.5)	68/242 (28.1)	29/88 (33.0)	<0.001

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
n	77	97	311	110	
ATOPY **	27/63 (42.9)	79/89 (88.8)	231/287 (80.5)	66/99 (66.7)	<0.001
Median number of sensitisations (range)	0 (0-5)	4 (0-6)	1 (0-6)	0.5 (0-6)	<0.001

*Sensitisation is defined as a positive skin prick test or positive specific IgE.

**Atopy is defined as sensitisation to one or more of the 6 aeroallergens listed. Figures differ from the baseline paediatric and adult papers[142,143] due to processing of additional IgE samples after publication of the baseline papers.

Table 30 Pairwise Comparisons of the Prevalence of Allergic Sensitisation in the Severe UBIOPRED Cohorts

	SW vs SA	SW vs SAn	SW vs SAs/ex	SA vs SAn	SA vs SAs/ex	SAn vs SAs/ex
Atopy % vs % (p-value)	42.9 vs 88.8 (<0.001)	42.9 vs 80.5 (<0.001)	42.9 vs 66.7 (0.003)	88.8 vs 80.5 (0.073)	88.8 vs 66.7 (<0.001)	80.5 vs 66.7 (0.005)
Number of sensitisations median vs median (p-value)	0 vs 4 (<0.001)	0 vs 1 (<0.001)	0 vs 1 (0.129)	4 vs 1 (<0.001)	4 vs 1 (<0.001)	1 vs 1 (0.084)

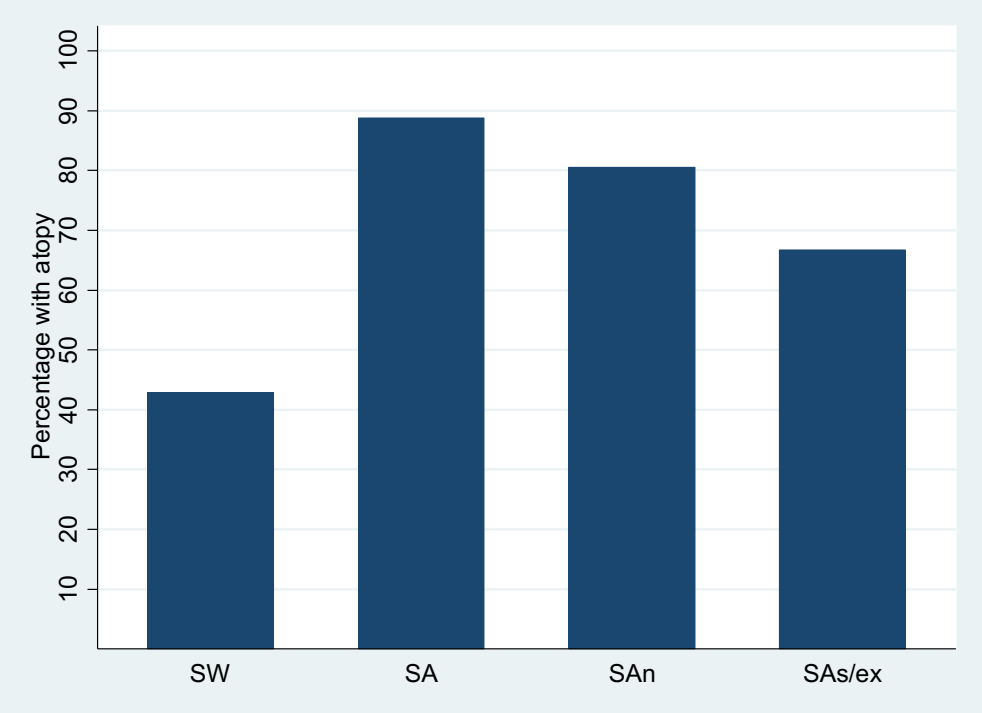

Figure 12 Bar Chart showing the Prevalence of Atopy in the Severe UBIOPRED Cohorts

Figure 13 Box Plots showing the Number of Allergens to which Participants in the Severe UBIOPRED Cohorts were Sensitised

Table 31 Prevalence of Allergic Sensitisation in the Mild to Moderate UBIOPRED Cohorts

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
Positive skin prick test (≥3mm)				
Tree pollen	4/47 (8.5)	16/34 (47.1)	23/49 (46.9)	<0.001
Grass pollen	7/47 (14.9)	26/36 (72.2)	41/63 (65.1)	<0.001
Dog	11/47 (23.4)	17/36 (47.2)	29/46 (63.0)	0.001
Cat	13/48 (27.1)	20/36 (55.6)	44/64 (68.8)	<0.001
House dust mite	6/47 (12.8)	22/36 (61.1)	40/60 (66.7)	<0.001
Mould	1/44 (2.3)	11/35 (31.4)	16/53 (30.2)	0.001
Positive specific IgE (≥0.35 kU/l)				
Tree pollen	4/18 (22.2)	12/16 (75.0)	14/26 (53.9)	0.008
Grass pollen	7/17 (41.2)	13/17 (76.5)	29/39 (74.4)	0.041
Dog	6/18 (33.3)	14/17 (82.4)	22/37 (59.5)	0.013
Cat	8/19 (42.1)	13/16 (81.3)	21/37 (56.8)	0.062
House dust mite	4/19 (21.1)	13/15 (88.7)	26/38 (68.4)	<0.001
Mould	3/21 (14.3)	10/14 (71.4)	10/32 (31.3)	0.002
Sensitisation *				
Tree pollen	7/50 (14.0)	22/38 (57.9)	33/67 (49.3)	<0.001
Grass pollen	10/50 (20.0)	29/39 (74.4)	56/81 (69.1)	<0.001
Dog	15/52 (28.9)	23/39 (59.0)	47/76 (61.8)	0.001
Cat	15/52 (28.9)	24/39 (61.5)	53/80 (66.3)	<0.001
House dust mite	8/52 (15.4)	28/40 (70.0)	53/79 (67.1)	<0.001
Mould	4/48 (8.3)	16/38 (42.1)	24/73 (32.9)	0.001

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
n	54	43	88	
ATOPY **	20/48 (41.7)	35/39 (89.7)	78/87 (89.7)	<0.001
Median number of sensitisations (range)	0 (0-6)	4 (0-6)	3 (0-6)	<0.001

*Sensitisation is defined as a positive skin prick test or positive specific IgE.

**Atopy is defined as sensitisation to one or more of the 6 aeroallergens listed. Figures differ from the baseline paediatric and adult papers[142,143] due to processing of additional IgE samples after publication of the baseline papers.

Table 32 Pairwise Comparisons of the Prevalence of Allergic Sensitisation in the Mild to Moderate UBIOPRED Cohorts

	MMW vs MMA	MMW vs MMAn	MMA vs MMAn
Atopy % vs % (p-value)	41.7 vs 89.7 (<0.001)	41.7 vs 89.7 (<0.001)	89.7 vs 89.7 (0.988)
Number of sensitisations median vs median (p-value)	0 vs 4 (<0.001)	0 vs 3 (<0.001)	4 vs 3 (0.084)

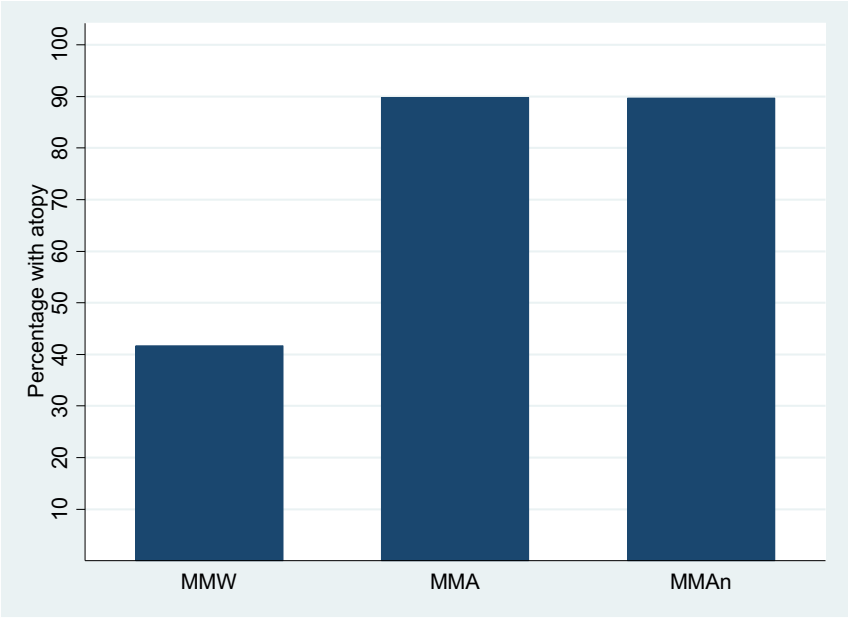

Figure 14 Bar Chart showing the Prevalence of Atopy in the Mild to Moderate UBIOPRED Cohorts

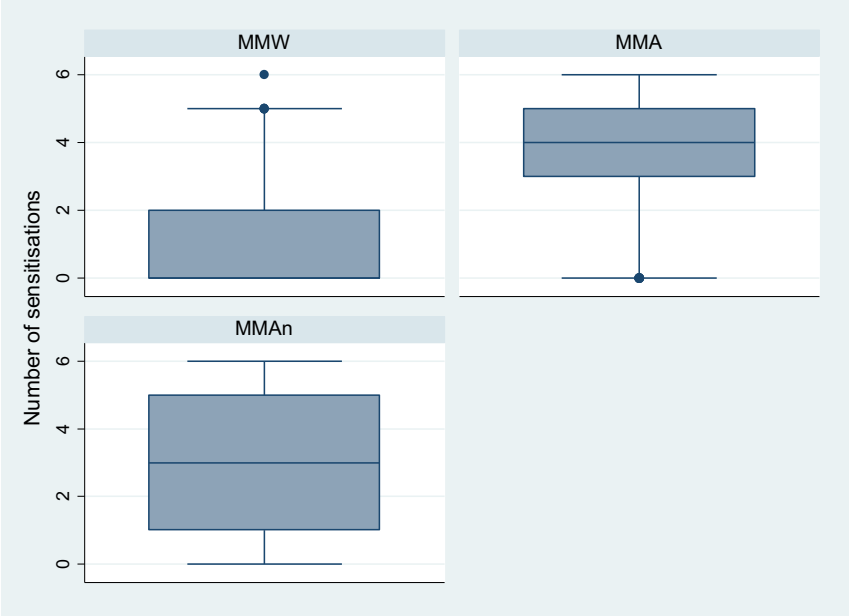

Figure 15 Box Plots showing the Number of Allergens to which Participants in the Mild to Moderate UBIOPRED Cohorts were Sensitised

5.5 Sensitisation versus Exposure

5.5.1 Sensitisation versus Exposure in the Severe Cohorts

13% of school aged participants with severe asthma were sensitised and exposed to cat compared to only 4% of preschool children with severe wheeze. For dog, the equivalent figures were 15% and 2%. The majority of preschool children with severe wheeze were neither exposed nor sensitised to cat or dog (72% for cat and 75% for dog). In adults, levels of exposure and sensitisation to cat and dog were higher than those for preschool children but lower than those in school aged children (Table 33, Figure 16 and Figure 17).

5.5.2 Sensitisation versus Exposure in the Mild to Moderate Cohorts

For cat, non-smoking adults with mild to moderate asthma were most likely to be sensitised and exposed (24%). This compares to 2% of preschool children with mild to moderate wheeze and 10% of school aged children with mild/moderate asthma. The prevalence of sensitisation and exposure to dog was, however, higher amongst school aged children with mild to moderate asthma (23%) than non-smoking adults with mild to moderate asthma (16%). Similar to the severe cohorts, the majority of preschool children were neither sensitised nor exposed to cat or dog (60% for cat and 70% for dog) (Table 34Table 33, Figure 18 and Figure 19).

Table 33 Relationship between Pet Sensitisation and Exposure in the Severe UBIOPRED Cohorts

	Preschool children (SW)	School aged children (SA)	Non-smoking adults (SAn)	Smokers and ex-smokers (SAs/ex)	p-value
Cat					
Sensitised and exposed	3/69 (4.4)	11/87 (12.6)	18/257 (7.0)	8/86 (9.3)	<0.001
Sensitised, not exposed	8/69 (11.6)	47/87 (54.0)	95/257 (37.0)	17/86 (19.8)	
Exposed, not sensitised	8/69 (11.6)	2/87 (2.3)	24/257 (9.3)	10/86 (11.6)	
Not exposed, not sensitised	50/69 (72.4)	27/87 (31.0)	120/257 (46.7)	51/86 (59.3)	
Dog					
Sensitised and exposed	1/67 (1.5)	13/88 (14.8)	26/249 (10.4)	7/89 (7.9)	<0.001
Sensitised, not exposed	7/67 (10.4)	42/88 (47.7)	72/249 (28.9)	20/89 (22.5)	
Exposed, not sensitised	9/67 (13.4)	10/88 (11.4)	26/249 (10.4)	22/89 (24.7)	
Not exposed, not sensitised	50/67 (74.6)	23/88 (26.1)	125/249 (50.2)	40/89 (44.9)	

Values represent n/N (%).

Sensitisation is defined as a positive skin prick test (≥3mm) or a positive specific IgE (≥0.35 kU/l).

Exposure refers to the presence of a cat/dog inside the home.

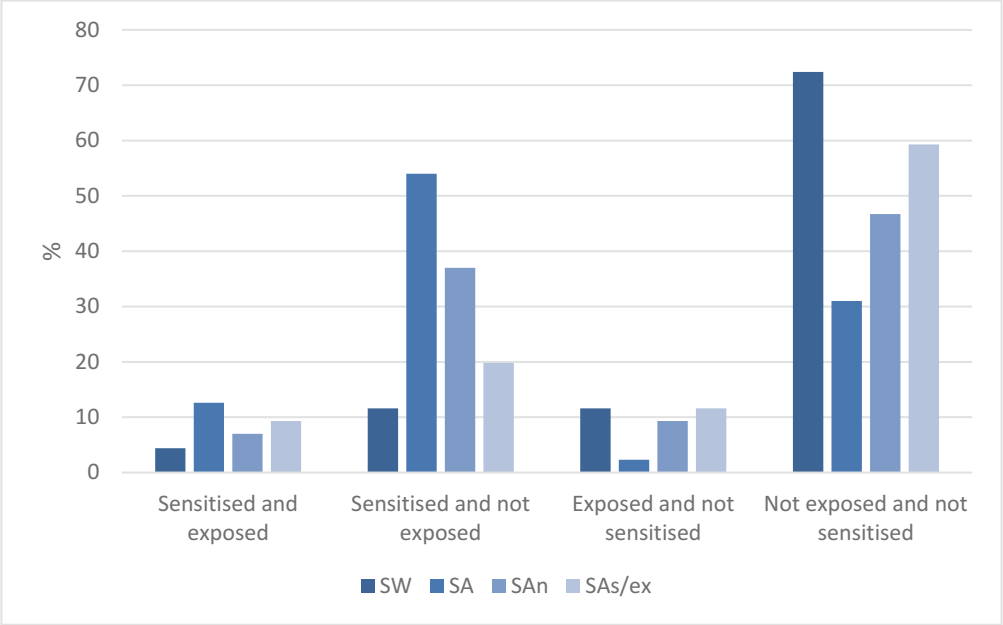

Figure 16 Bar Chart showing the Prevalence of Sensitisation and Exposure to Cat in the Severe UBIOPRED Cohorts

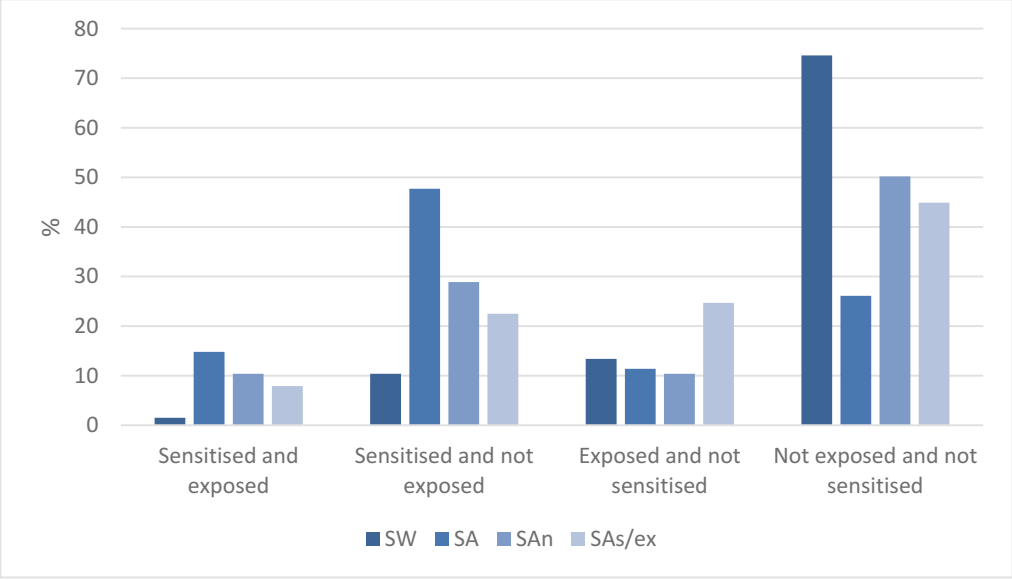

Figure 17 Bar Chart showing the Prevalence of Sensitisation and Exposure to Dog in the Severe UBIOPRED Cohorts

Table 34 Relationship between Pet Sensitisation and Exposure in the Mild to Moderate UBIOPRED Cohorts

	Preschool children (MMW)	School aged children (MMA)	Non-smoking adults (MMAn)	p-value
Cat				
Sensitised and exposed	1/52 (1.9)	4/39 (10.3)	19/80 (23.8)	<0.001
Sensitised, not exposed	14/52 (26.9)	20/39 (51.3)	35/80 (43.8)	
Exposed, not sensitised	6/52 (11.5)	3/39 (7.7)	7/80 (8.8)	
Not exposed, not sensitised	31/52 (59.6)	12/39 (30.8)	19/80 (23.8)	
Dog				
Sensitised and exposed	1/52 (1.9)	9/39 (23.1)	12/76 (15.8)	<0.001
Sensitised, not exposed	14/52 (26.9)	14/39 (35.9)	35/76 (46.1)	
Exposed, not sensitised	0/52 (0.0)	4/39 (10.3)	5/76 (6.6)	
Not exposed, not sensitised	37/52 (71.2)	12/39 (30.8)	24/76 (31.6)	

Values represent n/N (%).

Sensitisation is defined as a positive skin prick test (≥3mm) or a positive specific IgE (≥0.35 kU/l).

Exposure refers to the presence of a cat/dog inside the home.

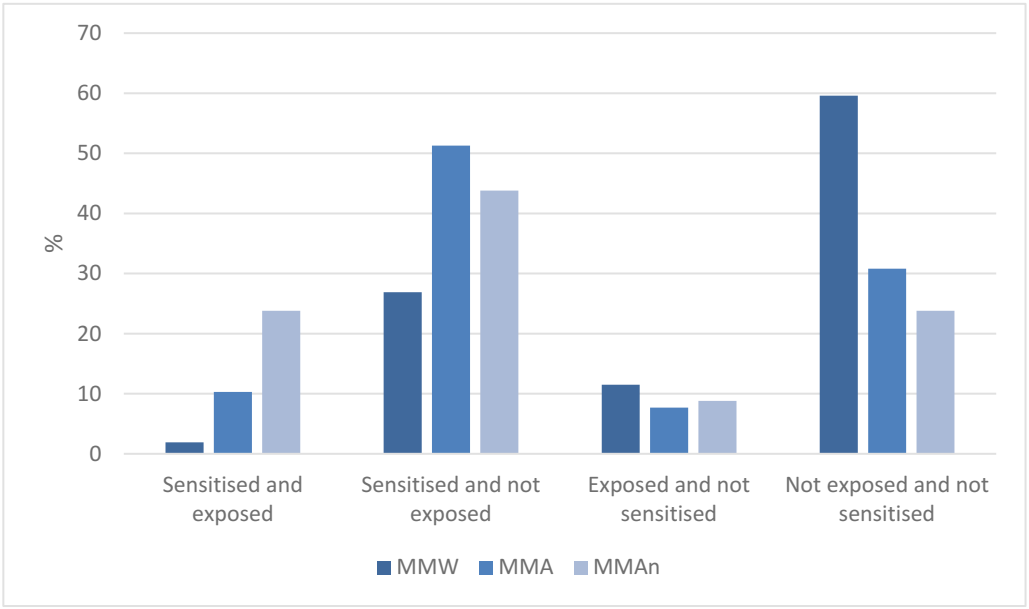

Figure 18 Bar Chart showing the Prevalence of Sensitisation and Exposure to Cat in the Mild to Moderate UBIOPRED Cohorts

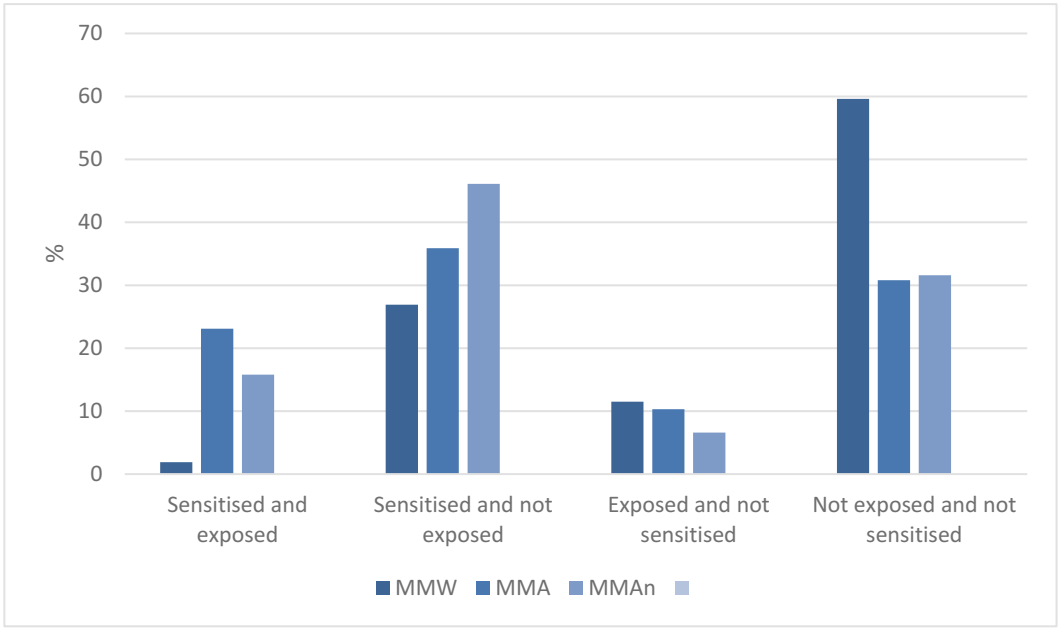

Figure 19 Bar Chart showing the Prevalence of Sensitisation and Exposure to Dog in the Mild to Moderate UBIOPRED Cohorts

5.6 Comparing Participants with Severe and Mild to Moderate Disease

When comparing the prevalence of allergic manifestations between participants of the same age with severe and mild to moderate disease, few significant differences were seen (Table 35 and Table 36).

For preschool children, the prevalence of hay fever was higher amongst those with severe wheeze compared to those with mild to moderate wheeze (44 vs 26%, p=0.043). The prevalences of eczema, allergic rhinitis, food allergy and atopy were however similar between the two groups. A higher proportion of children in the severe wheeze cohort were sensitised to house dust mite compared to the mild to moderate wheeze cohort (15 vs 31%, p=0.044). Sensitisation to dog was, however, more common amongst children with mild to moderate wheeze (28.9% vs 11.9% for children with severe wheeze, p=0.021).

For school aged children with asthma, there were no differences between those with mild to moderate and severe disease.

Non-smoking adults with mild to moderate asthma were more likely to be atopic than non-smoking adults with severe asthma (90 vs 81%, p=0.048) and smokers/ex-smokers with severe asthma (90 vs 67%, p=0.001). The median number of sensitisations was also higher in non-smoking adults with mild to moderate asthma than the other adult cohorts. Specifically, non-smoking adults with mild to moderate asthma were more likely to be sensitised to grass pollen, dog, cat and house dust mite than non-smoking adults with severe asthma and smokers/ex-smokers with severe asthma. The prevalences of eczema, allergic rhinitis, hay fever and food allergy did not differ significantly between adults with severe and mild to moderate asthma.

Table 35 Prevalence of Allergic Diseases according to Asthma/Wheeze Severity

	Adult Asthma Cohorts					School Aged Asthma Cohorts			Preschool Wheeze Cohorts		
	MMAn	**SAn**	**p-value***	**SAs/ex**	**p-value****	**MMA**	**SA**	**p-value**	**MMW**	**SW**	**p-value**
Diagnosed eczema	25/87 (28.7)	107/308 (34.7)	0.294	31/108 (28.7)	0.996	28/43 (65.1)	77/97 (79.4)	0.072	32/54 (59.3)	42/74 (56.8)	0.777
Diagnosed allergic rhinitis	42/82 (51.2)	164/29 (56.4)	0.409	44/108 (40.7)	0.151	29/41 (70.7)	61/95 (64.2)	0.461	11/51 (21.6)	22/69 (31.9)	0.211
Diagnosed hay fever	46/85 (54.1)	135/298 (45.3)	0.151	51/107 (47.7)	0.374	33/43 (76.7)	75/93 (80.7)	0.601	13/50 (26.0)	30/68 (44.1)	0.043
Possible food allergy	5/86 (5.8)	25/286 (8.7)	0.382	3/105 (2.9)	0.310	12/41 (29.3)	28/92 (30.4)	0.892	8/48 (16.7)	7/74 (9.5)	0.236
Highly likely food allergy	1/86 (1.2)	8/286 (2.8)	0.387	2/105 (1.9)	0.682	8/41 (19.5)	15/92 (16.3)	0.651	2/48 (4.2)	2/74 (2.7)	0.657
Atopy	78/87 (89.7)	231/287 (80.5)	0.048	66/99 (66.6)	<0.001	35/39 (89.7)	79/89 (88.8)	0.870	20/48 (41.7)	27/63 (42.9)	0.900

Data are presented as n/N (%). p-values were calculated using the Chi-squared test. *MMAn vs SAn. **MMAn vs SAs/ex.

MMAn= Non-smoking adults with mild to moderate asthma; SAn= Non-smoking adults with severe asthma; SAs/ex= Smokers and ex-smokers with severe asthma.
MMA= School aged children with mild to moderate asthma; SA= School aged children with severe asthma; MMW= Preschool children with mild to moderate wheeze; SW= Preschool children with severe wheeze.

Possible food allergy is defined as symptoms of food allergy plus evidence of sensitisation to the triggering food.
Highly likely food allergy is defined as symptoms of food allergy plus a ≥5mm skin prick test wheal or a specific IgE level ≥ 10.0 kU/l.

Table 36 Prevalence of Allergic Sensitisation and Atopy according to Asthma/Wheeze Severity

	Adult Asthma Cohorts					School Aged Asthma Cohorts			Preschool Wheeze Cohorts		
	MMAn	**SAn**	**p-value***	**SAs/ex**	**p-value****	**MMA**	**SA**	**p-value**	**MMW**	**SW**	**p-value**
Tree pollen	33/67 (49.6)	90/208 (43.3)	0.392	28/82 (34.1)	0.062	22/38 (57.9)	46/85 (54.1)	0.697	7/50 (14.0)	9/65 (13.9)	0.981
Grass pollen	56/81 (69.1)	119/260 (45.8)	<0.001	32/92 (34.8)	<0.001	30/40 (75.0)	57/90 (63.3)	0.192	10/50 (20.0)	9/69 (13.0)	0.307
Dog	47/76 (61.8)	98/251 (39.0)	<0.001	27/89 (30.3)	<0.001	23/39 (59.0)	55/88 (62.5)	0.707	15/52 (28.9)	8/67 (11.9)	0.021
Cat	54/81 (66.7)	115/260 (44.2)	<0.001	25/86 (29.1)	<0.001	24/39 (61.5)	58/87 (66.7)	0.577	15/52 (28.9)	11/6 (15.9)	0.087
House dust mite	53/79 (67.1)	143/274 (52.2)	0.019	41/96 (42.7)	0.001	28/40 (70.0)	59/90 (65.6)	0.619	8/52 (15.4)	21/67 (31.3)	0.044
Mould	24/73 (32.9)	68/24 (28.1)	0.431	29/88 (33.0)	0.992	16/38 (42.1)	37/87 (42.5)	0.965	4/48 (8.3)	5/63 (7.9)	0.940
ATOPY	78/87 (89.7)	231/287 (80.5)	0.048	66/99 (66.6)	<0.001	35/39 (89.7)	79/89 (88.8)	0.870	20/48 (41.7)	27/63 (42.9)	0.900
Median number of sensitisations (range)	3 (0-6)	1 (0-6)	<0.001	0.5 (0-6)	<0.001	4 (0-6)	4 (0-6)	0.924	0 (0-6)	0 (0-5)	0.635

Data are presented as n/N (%) unless otherwise stated. p-values were calculated using the Chi-squared test. *MMAn vs SAn. **MMAn vs SAs/ex.

MMAn= Non-smoking adults with mild to moderate asthma; SAn= Non-smoking adults with severe asthma; SAs/ex= Smokers and ex-smokers with severe asthma.

MMA= School aged children with mild to moderate asthma; SA= School aged children with severe asthma; MMW= Preschool children with mild to moderate wheeze; SW= Preschool children with severe wheeze.

Sensitisation is defined a positive skin prick test (≥3mm wheal) or a positive specific IgE (≥ 0.35 kU/l).

Atopy is defined as sensitisation to one or more of the 6 aeroallergens listed.

Chapter 6: UBIOPRED Results- Exacerbations

6.1 Participants

6.1.1 Paediatric Participants

Of the 282 paediatric participants recruited, 99 were school aged children with severe asthma (SA) and 81 were preschool children with severe wheeze (SW). Baseline data were available for analysis in 97 and 77 of these children respectively. 82% of the school aged children with severe asthma and 83% of preschool children with severe wheeze were followed up (Figure 20).

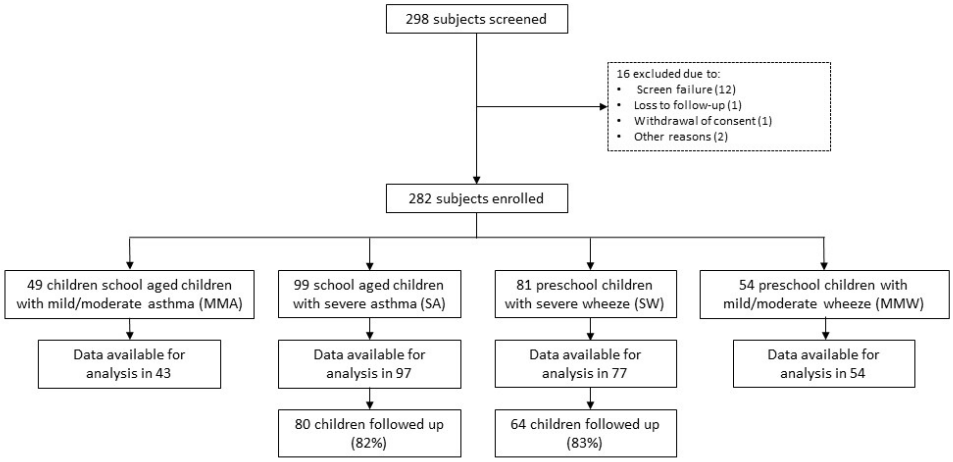

Figure 20 Consort Diagram showing Follow up in the Severe Paediatric UBIOPRED Cohorts

The baseline characteristics of all participants in the SA and SW cohorts and and the baseline characteristics of those who were followed up are described in Table 37.

Table 37 Baseline Characteristics of Children in the Severe Cohorts with and without Follow Up Data

	Preschool Children with Severe wheeze (SW)		School Aged Children with Severe asthma (SA)	
	All participants	**Participants with follow up data**	**All participants**	**Participants with follow up data**
Demographic details				
Female	27/77 (35.1)	24/64 (37.5)	46/97 (47.4)	40/80 (50.0)
Age (years)	3.56 ± 0.14 (n=77)	3.5 ± 0.24 (n=64)	12.21 ± 0.31 (n=97)	12.1 ± 0.32 (n=80)
Caucasian	62/77 (80.5)	52/64 (81.3)	74/97 (76.3)	60/80 (75.0)
Anthropometry				
Height (cm)	102.88 ± 1.13 (n=76)	102.76 ± 1.23 (n=63)	152.82 ± 1.65 (n=97)	152.05 ± 1.88 (n=80)
Height z-score	1.14 ± 0.16 (n=76)	1.17 ± 0.16 (n=63)	0.68 ± 0.34 (n=97)	0.42 ± 0.12 (n=80)
Weight (kg)	17.63 ± 0.48 (n=77)	17.51 ± 0.54 (n=64)	51.74 ± 1.85 (n=97)	50.89 ± 2.07 (n=80)
Weight z-score	0.94 ± 0.14 (n=77)	0.93 ± 0.15 (n=64)	1.14 ± 0.21 (n=97)	0.99 ± 0.14 (n=80)
BMI (kg/m^2)	16.56 ± 0.25 (n=76)	16.48 ± 0.27 (n=63)	21.52 ± 0.5 (n=97)	21.35 ± 0.56 (n=80)
BMI z-score	0.26 ± 0.15 (n=76)	0.19 ± 0.16 (n=63)	0.99 ± 0.13 (n=97)	0.95 ± 0.14 (n=80)
Asthma history				
Age at diagnosis (years)	1.74 ± 0.12 (n=73)	1.77 ± 0.12 (n=60)	3.25 ± 0.27 (n=93)	3.14 ± 0.30 (n=77)
ICU admission ever	9/77 (11.7)	6/64 (9.4)	9/97 (19.6)	15/80 (18.8)`
ICU admission in past year	6/77 (7.8)	5/64 (7.8)	5/97 (5.2)	3/80 (3.8)
Number of exacerbations in previous year	4 (1-6) (n=77)	3 (1-6) (n=64)	3 (2-5) (n=97)	4 (2-5) (n=80)
Reported triggers for respiratory symptoms				
Respiratory infections	77/77 (100.0)	64/64 (100.0)	91/96 (94.8)	74/79 (93.7)
Pets	14/60 (23.3)	10/51 (19.6)	62/92 (67.4)	53/76 (69.7)
Exercise	58/74 (78.4)	47/61 (77.1)	86/96 (89.6)	73/79 (92.4)
Cold air	61/72 (84.7)	49/59 (83.1)	79/97 (81.4)	67/80 (83.8)
Air pollutants	18/55 (32.7)	13/47 (27.7)	55/85 (64.7)	46/70 (65.7)
Stress	24/63 (38.1)	18/52 (34.6)	55/92 (59.8)	44/75 (58.7)
Pollens	34/65 (52.3)	24/53 (45.3)	76/93 (81.7)	62/76 (81.6)
Other medical problems				
Diagnosed hay fever	30/68 (44.1)	23/57 (40.4)	75/93 (80.7)	63/77 (81.8)
Diagnosed allergic rhinitis	22/69 (31.9)	17/58 (29.3)	61/95 (64.2)	51/78 (65.4)
Diagnosed eczema	42/74 (56.8)	35/62 (56.5)	77/97 (79.4)	63/80 (78.8)
Food allergy	7/74 (9.5)	7/62 (11.3)	28/92 (30.4)	22/75 (29.3)
Allergic sensitisation				
Grass pollen	9/69 (13.0)	7/57 (12.3)	57/90 (63.3)	48/76 (63.2)
Tree pollen	9/65 (13.9)	7/55 (12.7)	46/85 (54.1)	40/71 (56.3)
Dog	8/67 (11.9)	8/56 (14.3)	55/88 (63.5)	46/74 (62.2)
Cat	11/69 (15.9)	10/57 (17.5)	58/87 (66.7)	46/73 (63.0)
House dust mite	21/67 (31.3)	19/56 (33.9)	59/90 (65.6)	49/75 (65.3)
Mould	5/63 (7.9)	4/53 (7.6)	37/87 (42.5)	33/73 (45.2)
Atopy	27/63 (42.9)	24/53 (45.3)	79/89 (88.8)	65/74 (87.8)

	Preschool Children with Severe wheeze (SW)		School Aged Children with Severe asthma (SA)	
	All participants	**Participants with follow up data**	**All participants**	**Participants with follow up data**
Spirometry				
FEV_1 % predicted	104.34 ± 3.21 (n=19)	103.91 ± 3.04 (n=16)	88.68 ± 2.15 (n=96)	88.97 ± 2.48 (n=80)
FEV_1 z-score	0.33 ± 0.24 (n=19)	0.29 ± 0.22 (n=16)	-0.92 ± 0.18 (n=96)	-0.89 ± 0.20 (n=96)
FVC % predicted	107.99 ± 3.5 (n=19)	107.56 ± 2.66 (n=16)	102.15 ± 1.65 (n=96)	102.05 ± 1.88 (n=80)
FVC z-score	0.55 ± 0.25 (n=19)	0.53 ± 0.19 (n=16)	0.16 ± 0.14 (n=96)	0.15 ± 0.16 (n=80)
FEV_1/FVC ratio	0.91 ± 0.02 (n=19)	0.91 ± 0.02 (n=16)	0.77 ± 0.01 (n=96)	0.77 ± 0.01 (n=80)
Asthma related quality of life, asthma control and medication adherence				
Paediatric Asthma Quality of Life Questionnaire (PAQLQ)				
PAQLQ total score	NA	NA	4.77 ± 0.15 (n=91)	4.84 ± 0.17 (n=75)
PAQLQ total z-score	NA	NA	-0.22 ± 0.10 (n=91)	-0.16 ± 0.11 (n=75)
Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ)				
PACQLQ total score	4.27 ± 0.18 (n=77)	4.26 ± 0.20 (n=64)	NA	NA
PACQLQ total z-score	-0.46 ± 0.09 (n=77)	-0.46 ± 0.10 (n=64)	NA	NA
Combined quality of life z-score	-0.45 ± 0.09 (n=77)	-0.45 ±0.10 (n=64)	-0.22 ±0.10 (n=91)	-0.16 ± 0.11 (n=75)
Asthma Control Test (ACT)				
ACT total score	NA	NA	15.49 ± 0.63 (n=67)	15.83 ± 0.70 (n=54)
ACT total z-score	NA	NA	-0.25 ± 0.12 (n=67)	-0.18 ± 0.13 (n=54)
Childhood Asthma Control Test (C-ACT)				
C-ACT total score	15.2 ± 0.79 (n=41)	14.81 ± 0.95 (n=32)	16.38 ± 0.98 (n=29)	16.58 ± 1.01 (n=26)
C-ACT total z-score	-0.47 ± 0.13 (n=41)	-0.52 ± 0.15 (n=32)	-0.26 ± 0.16 (n=29)	-0.24 ± 0.17 (n=26)
Combined asthma control z-score	-0.47 ± 0.13 (n=41)	-0.52 ± 0.15 (n=32)	-0.26 ± 0.10 (n=95)	-0.21 ± 0.10 (n=79)
Medication Adherence Report Scale (MARS) total score	22.85 ± 0.26 (n=73)	23.02 ± 0.29 (n=60)	22.76 ± 0.23 (n=94)	22.77 ± 0.26 (n=77)

Figures represent n/N (%), mean ± SE or median (interquartile range) unless otherwise stated.

Sensitisation is defined as a positive skin prick test (≥3mm wheal) or a positive specific IgE (≥ 0.35 kU/l).
Food allergy is defined as symptoms of food allergy plus evidence of sensitisation.
Atopy is defined as sensitisation to one or more of the aeroallergens listed.

A higher quality of life z-score is associated with a better quality of life.
A higher asthma control z-score is associated with better asthma control.

The denominators and hence percentages for diagnosed eczema, allergic rhinitis and hay fever differ from those reported in the baseline UBIOPRED papers[142,143] due to a data processing error when preparing the baseline papers.

6.1.1.1 Clinical Clusters

249 paediatric participants (92%) were assigned to a clinical cluster. The sizes of the clinical clusters ranged from 87 children (cluster 3) to 13 children (cluster 6) (Figure 21). The relative sizes of the clusters remained similar when considering only participants with severe wheeze/asthma. Follow up was highest in cluster 5 (89%) and lowest in cluster 1 (71%) (Figure 22).

6.1.1.2 ISAC Component Atopy Clusters

ISAC chip data were available in 236 paediatric participants (87%): 101 (37%) were not sensitised, 46 (17%) had multiple sensitisation (cluster 1), 42 (15%) were sensitised to house dust mite (cluster 2), 17 (6%) were sensitised to grass pollen (cluster 3) and 30 (11%) had miscellaneous sensitisation (cluster 4) (Figure 23). For participants with severe asthma/wheeze only, the proportion in each ISAC component atopy cluster was similar: 14% in cluster 1, 20% in cluster 2, 5% in cluster 3, 13% in cluster 4 and 13% in the not sensitised group. For all the ISAC component atopy clusters, more than 80% of participants were followed up (Figure 24).

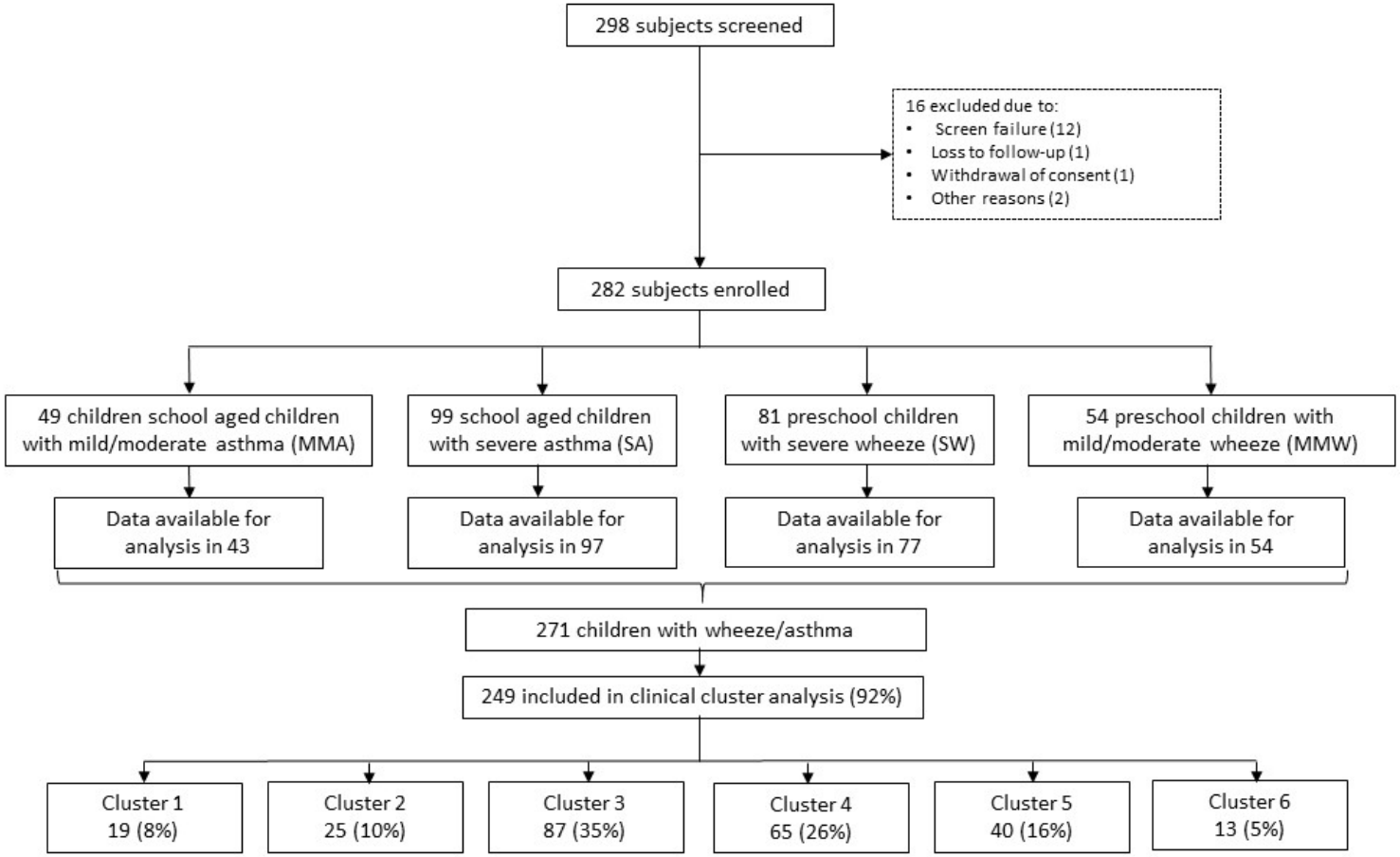

Figure 21 Consort Diagram showing the Number of Children from all Cohorts in each Clinical Cluster

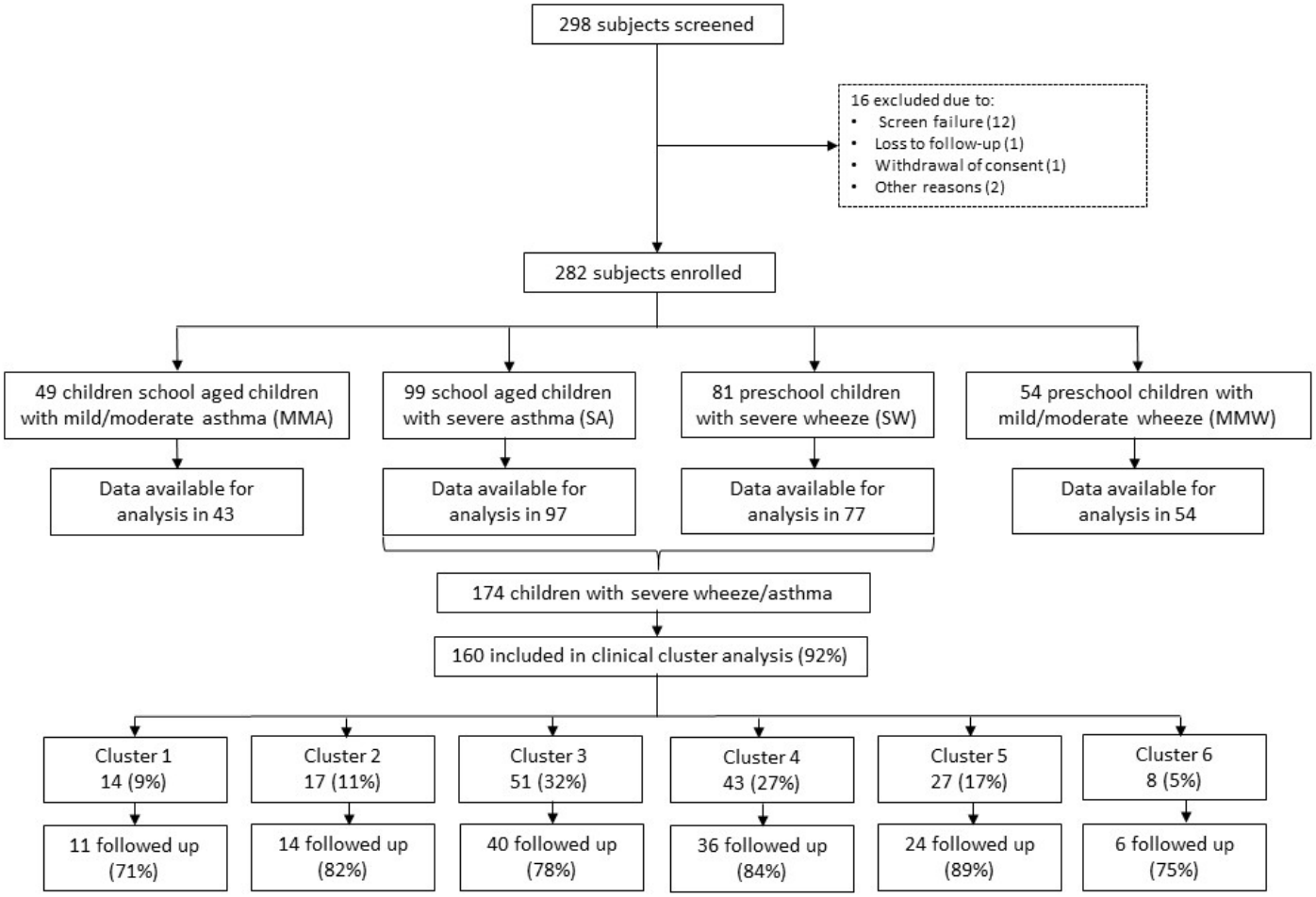

Figure 22 Consort Diagram showing the Number of Children from the Severe Cohorts in each Clinical Cluster

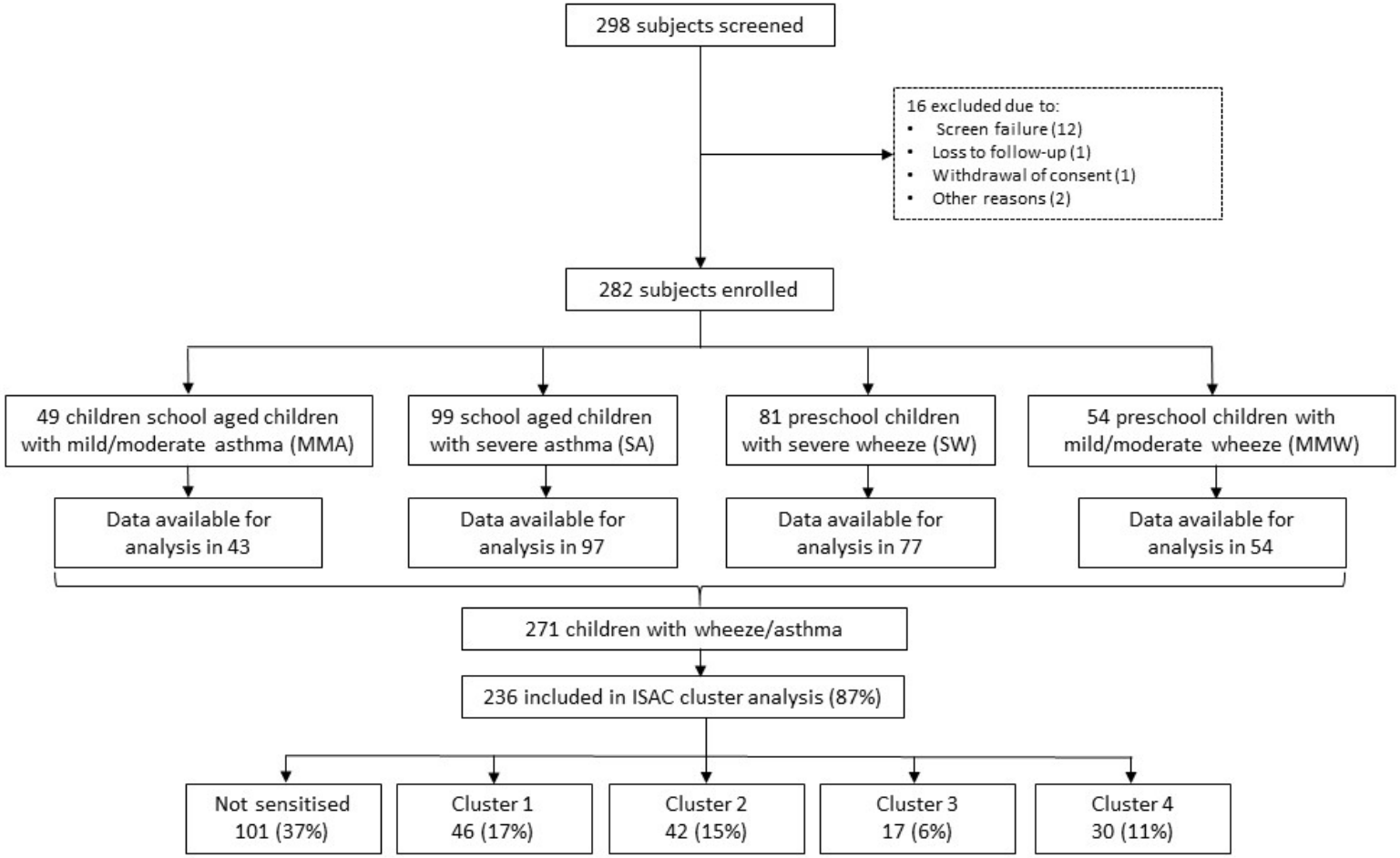

Figure 23 Consort Diagram showing the Number of Children from all Cohorts in each ISAC Component Atopy Cluster

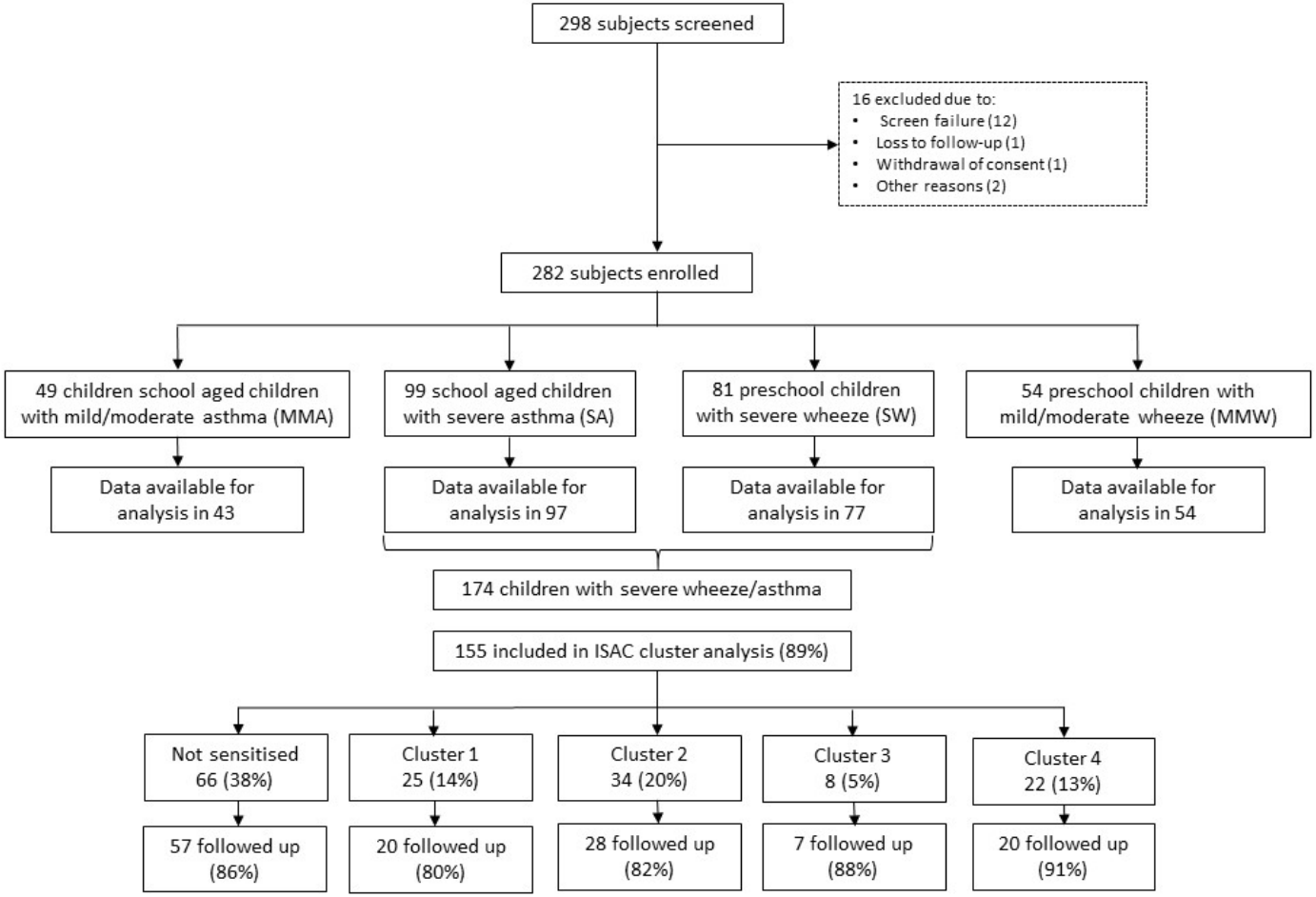

Figure 24 Consort Diagram showing the Number of Children from the Severe Cohorts in each ISAC Component Atopy Cluster

6.1.2 Adult Participants

A total of 610 adult participants were recruited: 311 non-smoking adults with severe asthma (SAn), 110 smokers/ex-smokers with severe asthma (SAs/ex), 88 non-smoking adults with mild to moderate asthma (MMAn) and 101 healthy controls (HC). 72% of those in the severe cohorts were followed up (Figure 25).

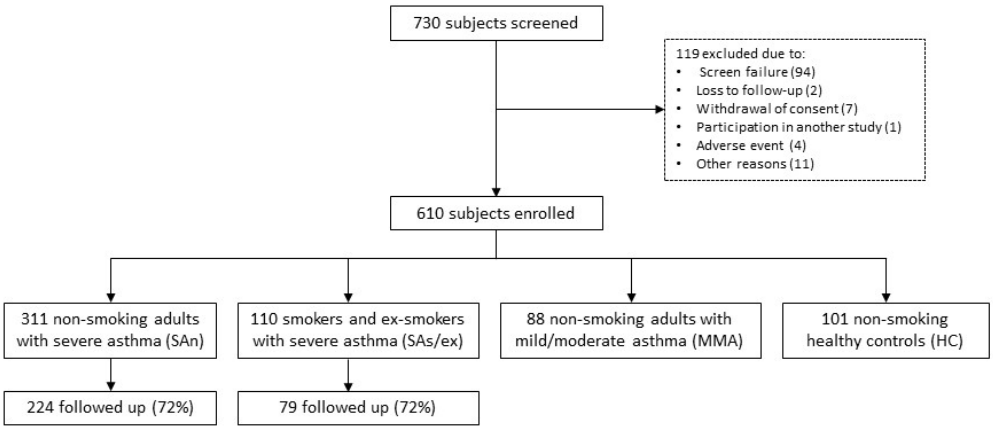

Figure 25 Consort Diagram showing Follow Up in the Severe Adult UBIOPRED Cohorts

As outlined in Table 38, the baseline characteristics of those followed up were similar to the baseline characteristrics of all participants in the SAn and SAs/ex cohorts.

Table 38 Baseline Characteristics of Adults in the Severe Cohorts with and without Follow Up Data

	Non-smoking Adults with Severe asthma (SAn)		Smokers and Ex-smokers with Severe Asthma (SAs/ex)	
	All participants	**Participants with follow up data**	**All participants**	**Participants with follow up data**
Demographic details				
Female	205/311 (65.9)	144/224 (64.3)	56/110 (50.9)	37/79 (46.8)
Age (years)	51.01 ± 0.8 (n=311)	51.70 ± 0.92 (n=224)	55.20 ± 1.25 (n=79)	54.51 ± 1.08 (n=110)
Caucasian	277/311 (89.1)	201/224 (89.7)	105/110 (95.5)	77/79 (97.5)
Anthropometry				
Height (cm)	166.46 ± 0.55 (n=311)	165 ± 0.65 (n=224)	169.21 ± 0.93 (n=110)	169.56 ± 1.17 (n=79)
Weight (kg)	80.68 ± 1.05 (n=311)	79.92 ± 1.16 (n=224)	84.80 ± 1.85 (n=110)	84.22 ± 2.09 (n=79)
BMI (kg/m^2)	29.11 ± 0.36 (n=311)	28.87 ± 0.40 (n=224)	29.59 ± 0.60 (n=110)	29.24 ± 0.64 (n=79)
Asthma history				
Age at diagnosis (years)	20 (7-38) (n=302)	20 (6-37) (n=218)	38 (20-48) (n=109)	35.5 (22-48) (n=78)
ICU admission ever	80/307 (26.1)	55/221 (24.9)	18/109 (16.5)	10/79 (12.7)
ICU admission in past year	13/310 (4.2)	4/110 (3.6)	9/223 (4.0)	1/79 (1.3)
Number of exacerbations in previous year	2 (1-3) (n=310)	2 (1-3) (n=224)	2 (1-4) (n=110)	2 (1-3) (n=79)
Reported triggers for respiratory symptoms				
Respiratory infections	271/304 (89.1)	200/219 (91.3)	92/110 (83.6)	66/79 (83.5)
Pets	139/287 (48.4)	100/205 (48.8)	34/105 (32.4)	26/75 (34.7)
Exercise	239/288 (83.0)	176/206 (85.4)	86/106 (81.1)	60/77 (77.9)
Cold air	237/304 (78.0)	177/219 (80.8)	53/104 (51.0)	35/76 (46.1)
Air pollutants	199/291 (68.4)	149/211 (70.6)	67/103 (65.1)	47/74 (63.5)
Stress	168/295 (57.0)	129/214 (60.3)	56/105 (53.3)	39/76 (51.3)
Pollens	184/293 (62.8)	135/211 (64.0)	49/104 (47.1)	34/74 (46.0)
Other medical problems				
Diagnosed hay fever	135/298 (45.3)	94/213 (44.1)	51/107 (47.7)	37/76 (48.7)
Diagnosed allergic rhinitis	164/291 (56.4)	115/209 (55.0)	44/108 (40.7)	32/78 (41.0)
Diagnosed eczema	107/308 (34.7)	76/221 (34.4)	31/108 (28.7)	19/78 (24.4)
Food allergy	25/286 (8.7)	16/205 (7.8)	3/105 (2.9)	1/76 (1.3)
Allergic sensitisation				
Grass pollen	119/260 (45.8)	88/188 (46.8)	32/92 (34.8)	25/66 (37.9)
Tree pollen	90/208 (43.3)	62/152 (40.8)	28/82 (34.2)	21/58 (36.2)
Dog	98/251 (39.0)	78/183 (42.6)	27/89 (30.3)	18/62 (29.0)
Cat	115/260 (44.2)	89/191 (46.6)	25/86 (29.1)	18/60 (30.0)
House dust mite	143/274 (52.2)	107/198 (54.0)	41/96 (42.7)	30/69 (43.5)
Mould	68/242 (28.1)	45/173 (26.0)	29/88 (33.0)	18/61 (29.5)
Atopy	231/287 (80.5)	166/208 (79.8)	66/99 (66.7)	47/71 (66.2)
Spirometry				
FEV_1 % predicted	67.5 ± 1.26 (n=308)	66.5 ± 1.45 (n=211)	67.2 ± 1.84 (n=110)	65.3 ± 2.21 (n=79)
FVC % predicted	87.2 ± 1.12 (n=308)	87.1 ± 1.31 (n=211)	89.7 ± 1.74 (n=110)	89.5 ± 2.07 (n=79)
FEV_1/FVC ratio	0.64 ± 0.01 (n=308)	0.63 ± 0.01 (n=221)	0.61 ± 0.01 (n=110)	0.60 ± 0.01 (n=79)

	Non-smoking Adults with Severe asthma (SAn)		Smokers and Ex-smokers with Severe Asthma (SAs/ex)	
	All participants	**Participants with follow up data**	**All participants**	**Participants with follow up data**
Asthma-related quality of life, asthma control and medication adherence				
Asthma Quality of Life Questionniare (AQLQ) total score	4.48 ± 0.07 (n=276)	4.49 ± 0.08 (n=214)	4.44 ± 0.13 (n=92)	4.53 ± 0.15 (n=72)
AQLQ z-score	-0.26 ± 0.05 (n=276)	-0.22 ± 0.06 (n=214)	-0.32 ± 0.09 (n=92)	-0.23 ± 0.11 (n=72)
Asthma Control Questionnaire (ACQ)				
Mean ACQ5	2.28 ± 0.07 (n=277)	2.28 ± 0.08 (n=216)	2.23 ± 0.12 (n=96)	2.09 ± 0.13 (n=74)
Mean ACQ7	2.67 ± 0.08 (n=277)	2.67 ± 0.08 (n=216)	2.62 ± 0.12 (n=96)	2.52 ± 0.13 (n=74)
ACQ-7 z-score	0.34 ± 0.05 (n=277)	0.34 ± 0.06 (n=216)	0.30 ± 0.09 (n=96)	0.23 ± 0.10 (n=74)
Medication Adherence Report Scale (MARS) total score	22.44 ± 0.14 (n=278)	22.43 ± 0.15 (n=214)	22.17 ± 0.29 (n=94)	22.05 ± 0.34 (n=75)

Figures represent n/N (%), mean ± SE or median (interquartile range) unless otherwise stated.

Sensitisation is defined as a positive skin prick test (≥3mm wheal) or a positive specific IgE (≥ 0.35 kU/l).
Food allergy is defined as symptoms of food allergy plus evidence of sensitisation.
Atopy is defined as sensitisation to one or more of the aeroallergens listed.

A higher AQLQ z-score is associated with a better quality of life.
A higher ACQ-7 z-score is associated with worse asthma control.

The denominators and hence percentages for diagnosed eczema, allergic rhinitis and hay fever differ from those reported in the baseline UBIOPRED papers[142,143] due to a data processing error when preparing the baseline papers.

6.1.2.1 Clinical Clusters

Data were available for clinical cluster analysis in 418 of the 509 adult participants with asthma (82%). 108 participants were assigned to cluster 1 (25%), 86 to cluster 2 (21%), 106 to cluster 3 (25%) and 118 to cluster 4 (28%) (Figure 26). For participants with severe asthma, 339 out of 421 were included in the clinical cluster analysis. 12% of these 339 participants belonged to cluster 1, 25% to cluster 2, 30% to cluster 3 and 33% to cluster 4. The proportion of participants followed up ranged from 68% for cluster 1 to 79% for cluster 4 (Figure 27).

6.1.2.2 ISAC Component Atopy Clusters

ISAC chip data were available in 491 of the 509 adult participants with asthma (96%). 49% were not sensitised with 9% of participants in cluster 1 (multiple sensitisation), 11% in cluster 2 (house dust mite sensitisation), 10% in cluster 3 (grass pollen sensitisation) and 21% in cluster 4 (miscellaneous sensitisation). ISAC chip data were also available in the majority of adults with severe asthma (96%) with a similar proportion of participants in each cluster (Figure 28). 70-71% of participants from clusters 1,2,4 and the non-sensitised group were followed up compared to 88% of participants from cluster 3 (Figure 29).

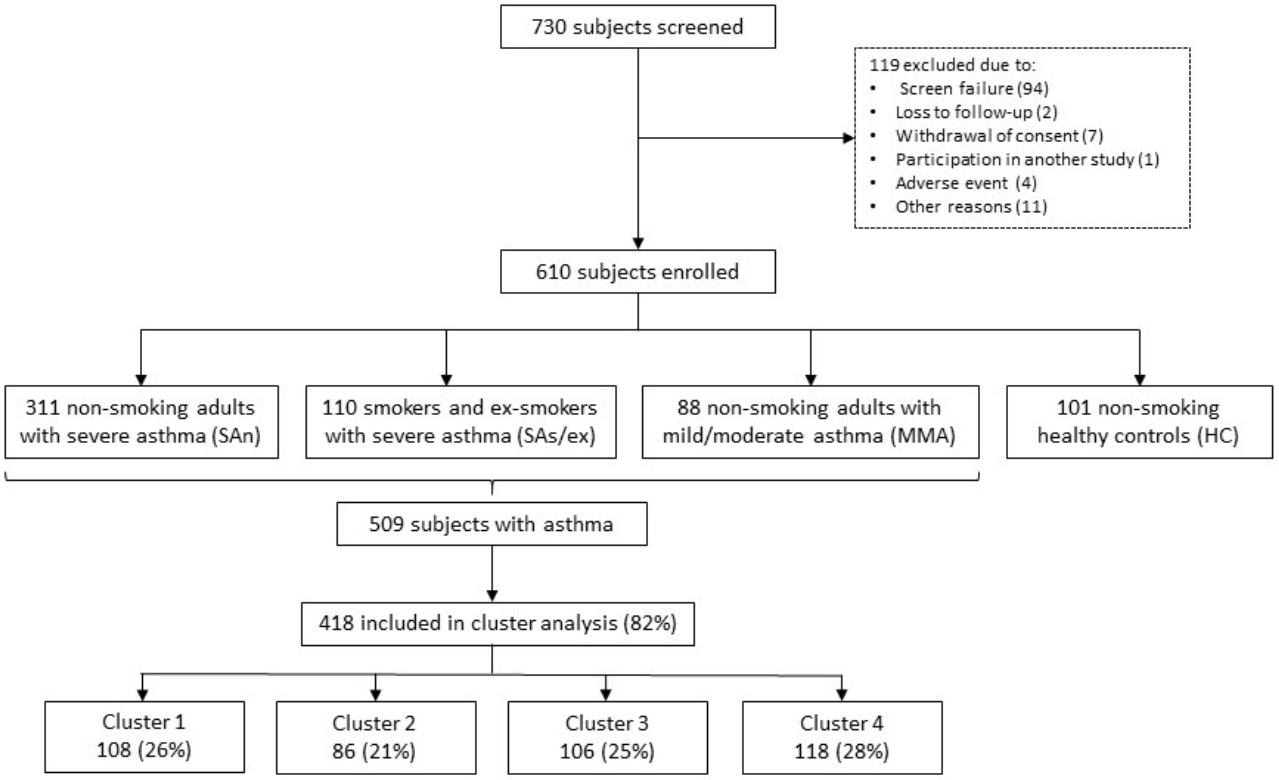

Figure 26 Consort Diagram showing the Number of Adults from all Cohorts in each Clinical Cluster

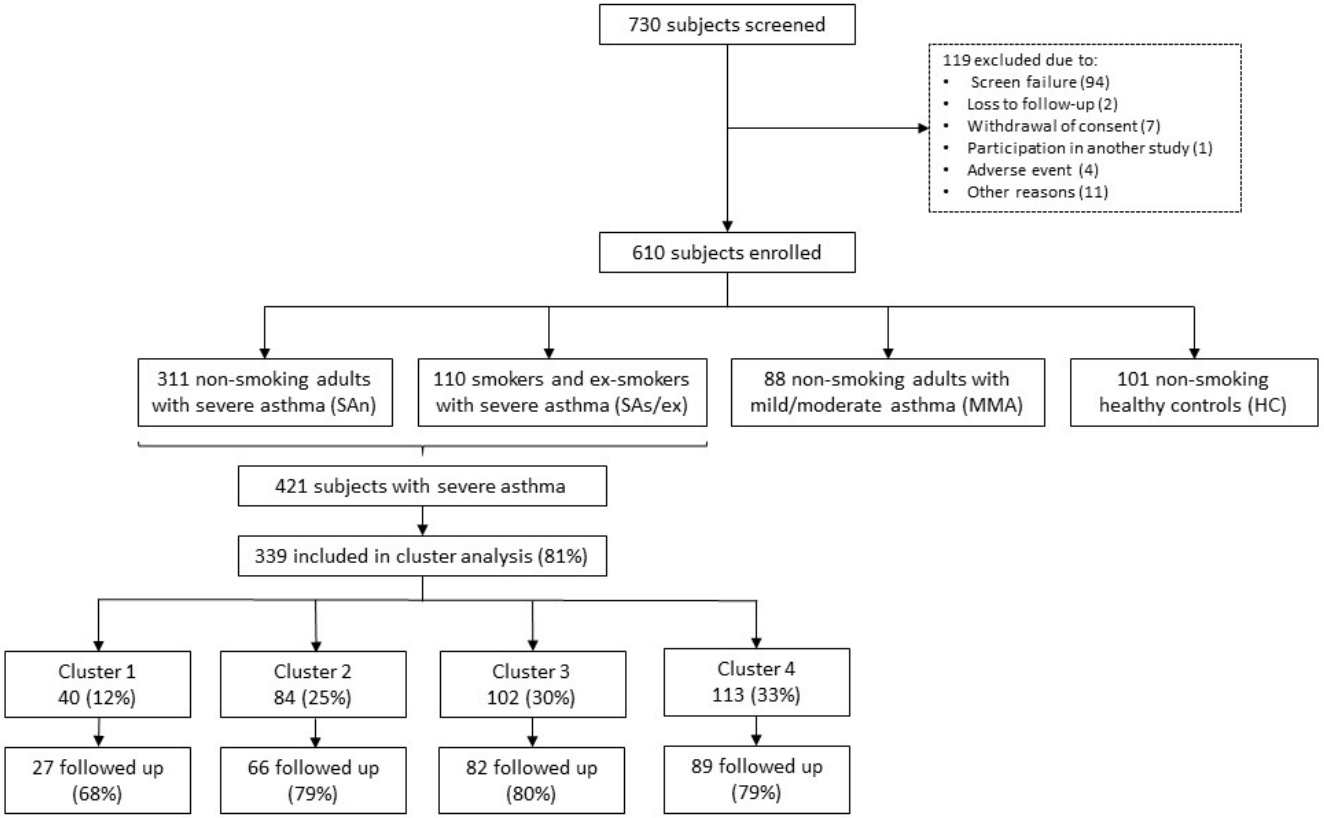

Figure 27 Consort Diagram showing the Number of Adults from the Severe Cohorts in each Clinical Cluster

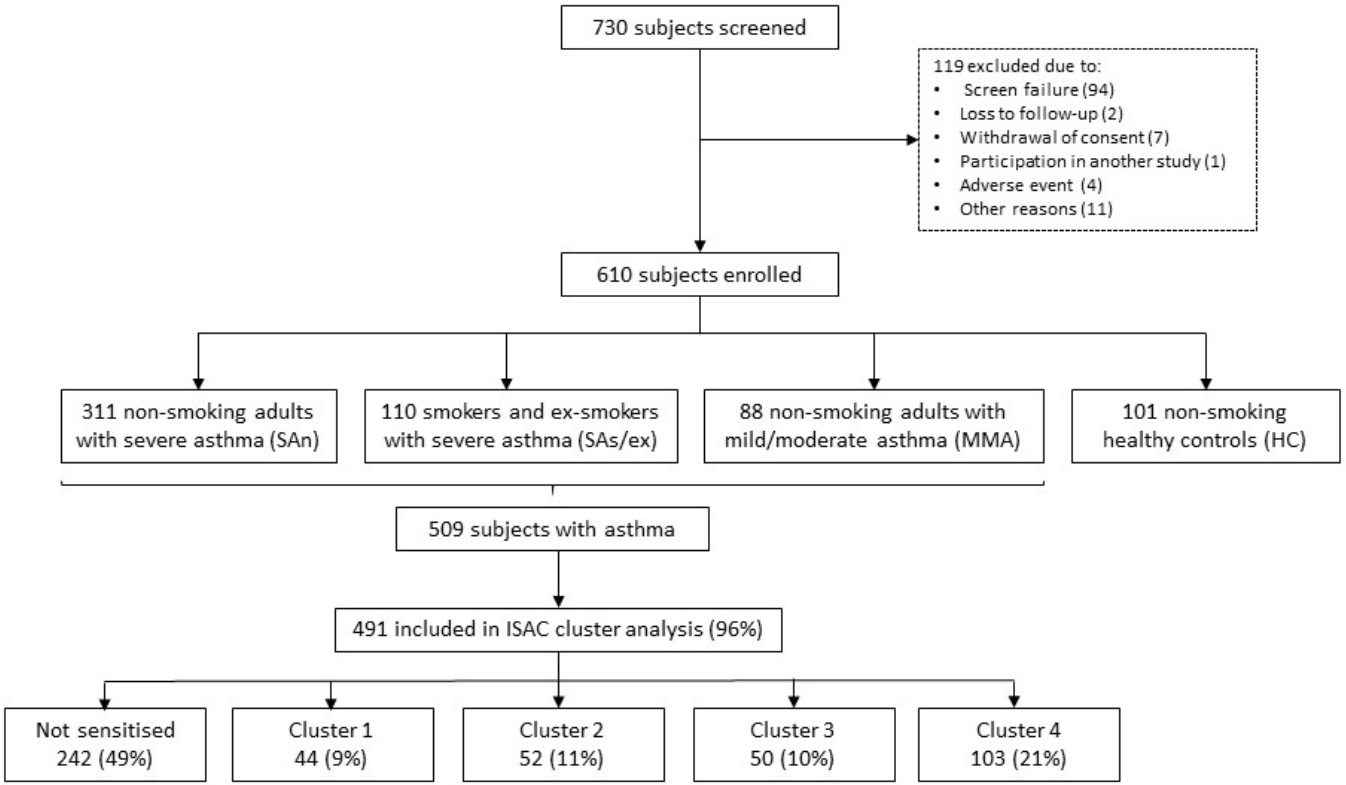

Figure 28 Consort Diagram showing the Number of Adult Participants in each ISAC Component Atopy Cluster

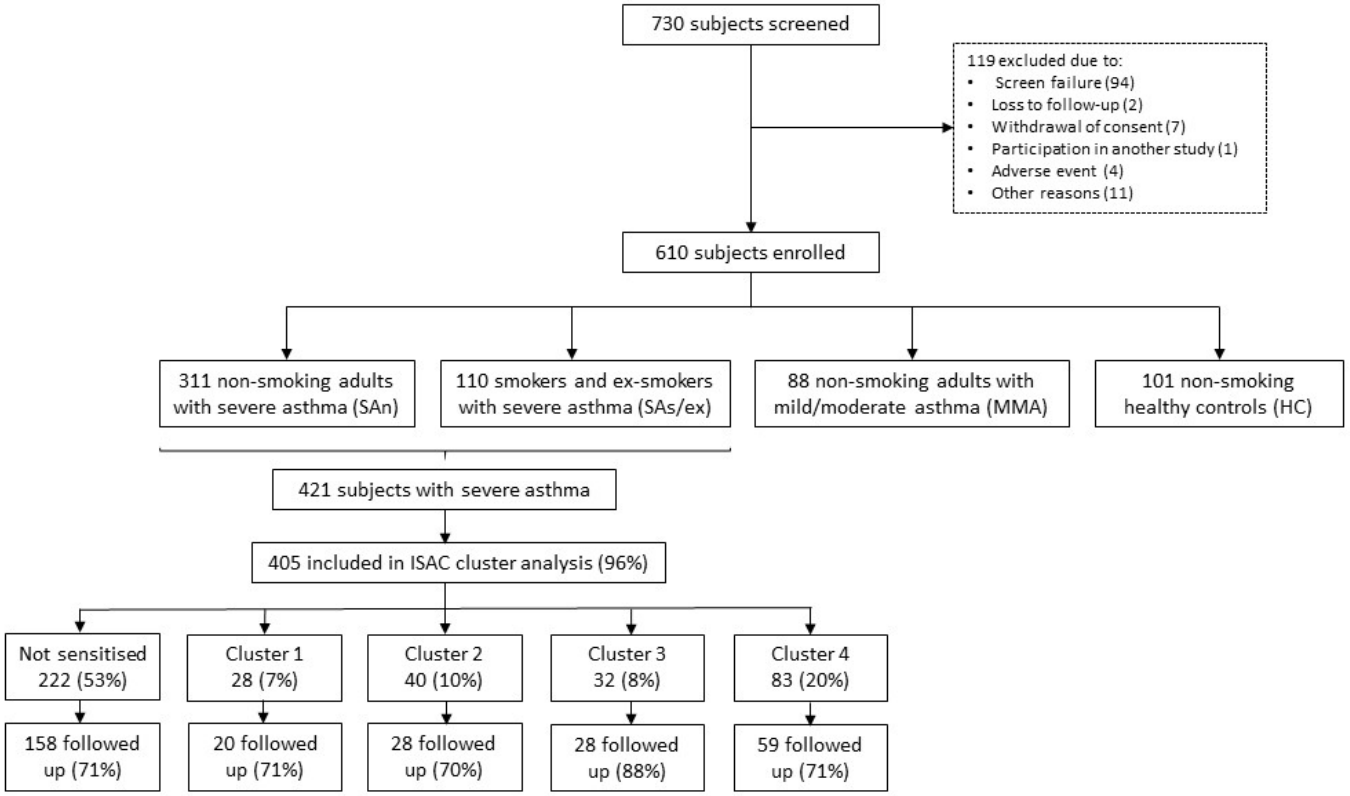

Figure 29 Consort Diagram showing the Number of Adults from the Severe Cohorts in each ISAC Component Atopy Cluster

6.2 Details of Exacerbations

6.2.1 Paediatric Participants

Overall 62% of children with severe asthma/preschool wheeze experienced one or more exacerbations during the study follow up period with 49% experiencing a severe exacerbation. Only 2% experienced a life-threatening exacerbation. A higher proportion of school aged children with asthma experienced an exacerbation compared to preschool children with severe wheeze. However, the difference between cohorts was not statistically significant for any type of exacerbation (Table 39). Exacerbation rates did not differ significantly between school aged children with severe asthma and preschool children with severe wheeze either (Table 39); the median rate of exacerbations in the preschool age cohort was 0.9 compared to 1.3 in the school aged cohort (p=0.125). For severe exacerbations only, the exacerbation rate was also higher in school aged children (0.7 versus 0 in preschool age children). However, this difference was not statistically significant (p=0.088).

Clinical clusters: The proportion of children experiencing any exacerbations during follow up ranged from 83.3% for cluster 6 to 55.3% for cluster 4. However, differences between clusters were not statistically significant. Rates of any exacerbations, severe exacerbations (both hospitalised and non-hospitalised) and life-threatening exacerbations did not differ between clusters either (Table 40). However, rates of moderate exacerbations did differ between clusters with pairwise comparisons demonstrating that cluster 2 had a higher exacerbation rate than clusters 4 and 5 (p=0.031 and p=0.019, respectively) and cluster 6 had a higher exacerbation rate than cluster 1 (p=0.025), cluster 4 (p=0.044) and cluster 5 (p=0.024) (Table 40).

ISAC component atopy clusters: The proportion of children experiencing any exacerbations was highest for cluster 1 (71%) and lowest for non-sensitised individuals (52.5%). However, differences between clusters were not statistically significant (Table 41). Similarly, exacerbation rates did not differ significantly between clusters with median exacerbation rates ranging from 0 to 2.1 and median rates of severe exacerbations ranging from 0-0.9.

Table 39 Details of Prospective Exacerbations in the Severe Paediatric Cohorts

	All children in severe cohorts	Preschool Children with Severe Wheeze (SW)	School Aged Children with Severe Asthma (SA)	p-value
	n=144	n=64	n=80	
Exacerbation rate (per year):				
All exacerbations	1 (0-3.1)	0.9 (0-2.4)	1.3 (0-3.9)	0.125
Moderate exacerbations	0 (0-0.8)	0 (0-0.8)	0 (0-0.8)	0.864
Severe exacerbations	0 (0-2)	0 (0-1.7)	0.7 (0-2.5)	0.088
- Non-hospitalised	0 (0-1.5)	0 (0-1)	0 (0-1.7)	0.081
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0)	0.386
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0.119
Any exacerbations (yes/no):				
All exacerbations	89/144 (61.8)	37/64 (57.8)	52/80 (65.0)	0.378
Moderate exacerbations	43/144 (29.9)	20/64 (31.3)	23/80 (28.8)	0.745
Severe exacerbations	71/144 (49.3)	27/64 (42.2)	44/80 (55.0)	0.126
- Non-hospitalised	60/144 (41.7)	22/64 (34.4)	38/80 (47.5)	0.112
- Hospitalised	29/144 (20.1)	11/64 (17.2)	18/80 (22.5)	0.430
Life-threatening	2/144 (2.0)	0/64 (0)	3/80 (3.8)	0.117

Figures represent medians (25^{th}-75^{th} centiles) and n/N (%).

Cohorts were compared using the Kruskal-Wallis/Chi-squared test.

Table 40 Details of Prospective Exacerbations in the Paediatric Clinical Clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	p-value
	n=11	n=15	n=40	n=38	n=25	n=6	
Exacerbation rate (per year):							
All exacerbations	3.4 (0-4.3)	2 (0.7-3)	1.0 (0-2.5)	0.8 (0-3.0)	0.8 (0-4.5)	0.9 (0.9-3.8)	0.652
Moderate exacerbations	0 (0-0)	0.8 (0-2)	0 (0-0.8)	0 (0-0)	0 (0-0)	0.9 (0-0.9)	0.037*
Severe exacerbations	0.9 (0-4)	0.7 (0-2.8)	0 (0-1.8)	0.3 (0-1.1)	0.8 (0-1.9)	0 (0-0)	0.762
- Non-hospitalised	0.9 (0-2.6)	0.7 (0-1)	0 (0-1)	0 (0-1)	0.4 (0-1.8)	0 (0-0)	0.450
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0.2)	0 (0-0)	0 (0-0)	0 (0-0)	0.949
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.716
Any exacerbations (yes/no):							
All exacerbations	7/11 (63.6)	12/15 (80.0)	24/40 (60.0)	21/38 (55.3)	14/24 (58.3)	5/6 (83.3)	0.525
Moderate exacerbations	2/11 (18.2)	8/15 (53.3)	13/40 (32.5)	9/38 (23.7)	4/24 (16.7)	4/6 (66.7)	0.045
Severe exacerbations	6/11 (54.6)	8/15 (53.3)	19/40 (47.5)	19/38 (50.0)	13/24 (54.2)	1/6 (16.7)	0.690
- Non-hospitalised	6/11 (54.5)	8/15 (53.3)	15/40 (37.5)	14/38 (36.8)	12/24 (50.0)	1/6 (16.7)	0.485
- Hospitalised	2/11 (8.2)	2/15 (13.3)	10/40 (25.0)	7/38 (18.4)	5/24 (20.8)	1/6 (16.7)	0.948
Life-threatening	0/11 (0)	0/15 (0.0)	1/40 (2.5)	2/38 (5.3)	0/24 (0)	0/6 (0)	0.718

Figures represent medians (25th-75th centiles) and n/N (%).

Clusters were compared using the Kruskal-Wallis/Chi-squared test.

*Pairwise comparisons of the group demonstrated that cluster 2 had a higher exacerbation rate than cluster 4 (p=0.031) and cluster 5 (p=0.019); cluster 6 had a higher exacerbation rate than cluster 1 (p=0.025), cluster 4 (p=0.044) and cluster 5 (p=0.024).

Table 41 Details of Prospective Exacerbations in the Paediatric ISAC Component Atopy Clusters

	Not sensitised	ISAC Cluster 1	ISAC Cluster 2	ISAC Cluster 3	ISAC Cluster 4	p-value
	n=59	n=21	n=28	n=7	n=20	
Exacerbation rate (per year):						
All exacerbations	0.9 (0-3)	1.5 (0-3.0)	1.0 (0-2.9)	0 (0-3.4)	2.1 (0-5.1)	0.445
Moderate exacerbations	0 (0-0.8)	0 (0-0.8)	0 (0-0.8)	0 (0-0)	0 (0-0.9)	0.547
Severe exacerbations	0 (0-2)	0 (0-1.7)	0.8 (0-1.3)	0 (0-3.4)	0.9 (0-4.1)	0.503
- Non-hospitalised	0 (0-1)	0 (0-1.3)	0 (0-1.0)	0 (0-1.7)	0.7 (0-2.7)	0.505
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-1.7)	0 (0-0.8)	0.112
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.151
Any exacerbations (yes/no):						
All exacerbations	31/59 (52.5)	15/21 (71.4)	19/28 (67.9)	3/7 (42.9)	14/20 (70.0)	0.295
Moderate exacerbations	16/59 (27.1)	7/21 (33.3)	9/28 (32.1)	0/7 (0.0)	6/20 (30.0)	0.508
Severe exacerbations	25/59 (42.4)	10/21 (47.6)	17/28 (60.7)	3/7 (42.9)	12/20 (60.0)	0.457
- Non-hospitalised	22/59 (37.3)	10/21 (47.6	12/28 (42.9)	2/7 (28.6)	11/20 (55.0)	0.606
- Hospitalised	9/59 (15.3)	2/21 (9.5)	6/28 (21.4)	3/7 (42.9)	7/20 (35.0)	0.116
Life-threatening	2/59 (3.4)	0/21 (0.0)	0/28 (0.0)	1/7 (14.3)	0/20 (0.0)	0.157

Figures represent medians (25^{th}-75^{th} centiles) and n/N (%).

Clusters were compared using the Kruskal-Wallis/Chi-squared test.

ISAC Cluster 1= Multiple sensitisation; ISAC Cluster 2= House dust mite sensitisation; ISAC Cluster 3= Grass pollen sensitisation; ISAC Cluster 4= Miscellaneous sensitisation.

6.2.2 Adult Participants

Overall, 67% of adult participants experienced one or more exacerbations during follow up with 57% of participants experiencing a severe exacerbation. Only 6 participants (2%) experienced a life-threatening exacerbation. The median rate of severe exacerbations was 1 per year for both non-smoking adults and smokers/ex-smokers (Table 42).

Clinical clusters: When taking all exacerbations into account, the rate of exacerbations per year did not differ significantly between clusters (Table 43). However, for severe exacerbations (both hospitalised and non-hospitalised) there were statistically significant differences between clusters. For severe hospitalised exacerbations, the rate of exacerbations was higher in clusters 3 and 4 (median 0.5 exacerbations per year) than clusters 1 and 2 (median 0 exacerbations per year). For severe non-hospitalised exacerbations, the exacerbation rate was higher in cluster 2 (median 0.5 exacerbations per year) than the other 3 clusters (median 0 exacerbations per year).

ISAC component atopy clusters: The proportion of adults experiencing one or more exacerbations during follow up differed significantly between ISAC component atopy clusters (Table 44). Rates of all exacerbations, severe hospitalised and severe non-hospitalised exacerbations also differed according to atopic sensitisation. For all exacerbations, the exacerbation rate was highest in non-sensitised individuals who had a median exacerbation rate of 1.7 exacerbations per year. This was significantly higher than an exacerbation rate of 1 exacerbation per year in individuals with miscellaneous sensitisation ($p=0.002$). The overall exacerbation rate in cluster 4 (miscellaneous sensitisation) was also lower than the exacerbation rate in cluster 2 (house dust mite sensitisation). The median rate of all severe exacerbations did not differ significantly between clusters. However, differences between clusters were seen when hospitalised and non-hospitalised severe exacerbations were considered separately (Table 44).

Table 42 Details of Prospective Exacerbations in the Severe Adult Cohorts

	All Adults in Severe Cohorts	Non-smoking Adults with Severe Asthma (SAn)	Smokers/Ex-smokers with Severe Asthma (SAs/ex)	p-value
	n=303	n=224	n=79	
Exacerbation rate (per year):				
All exacerbations	1 (0-2)	1 (0-3)	1 (0-2)	0.630
Moderate exacerbations	0 (0-0)	0 (0-0)	0 (0-0)	0.382
Severe exacerbations	1 (0-2)	1 (0-2)	1 (0-2)	0.888
- Non-hospitalised	0.7 (0-2)	0.9 (0-2)	0 (0-2)	0.889
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0)	0.329
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0.142
Any exacerbations (yes/no):				
All exacerbations	202/303 (66.7)	154/224 (68.8)	48/79 (60.8)	0.195
Moderate exacerbations	40/303 (13.2)	32/224 (14.3)	8/79 (10.1)	0.348
Severe exacerbations	172/303 (56.8)	130/224 (58.0)	42/79 (53.2)	0.452
- Non-hospitalised	153/303 (50.5)	116/224 (51.8)	37/79 (46.8)	0.449
- Hospitalised	33/303 (10.9)	27/224 (12.1)	6/79 (7.6)	0.274
Life-threatening	6/303 (2.0)	6/224 (2.7)	0/79 (0.0)	0.142

Figures represent medians (25^{th}-75^{th} centiles) and n/N (%).

Cohorts were compared using the Chi-squared/Kruskal-Wallis test.

Table 43 Details of Prospective Exacerbations in the Adult Clinical Clusters

	Cluster 1	Cluster 2	Cluster 3	Cluster 4	p-value
	n=27	n=66	n=82	n=89	
Exacerbation rate (per year):					
All exacerbations	1 (0-2)	1 (0-3)	1 (0-2.8)	2 (0-3)	0.244
Moderate exacerbations	0 (0-0.8)	0 (0-0)	0 (0-0)	0 (0-0)	0.208
Severe exacerbations	0 (0-1)	1 (0-2)	1 (0-2)	1 (0-3)	0.101
- Non-hospitalised	0 (0-1)	0.8 (0-2)	1 (0-2)	0.8 (0-2)	0.036 *
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.046 **
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.393
Any exacerbations (yes/no):					
All exacerbations	15/227 (55.6)	41/66 (62.1)	58/82 (70.7)	62/89 (69.7)	0.379
Moderate exacerbations	7/27 (25.9)	9/66 (13.6)	8/82 (9.8)	12/89 (13.5)	0.211
Severe exacerbations	10/27 (37.0)	35/66 (53.0)	49/82 (59.8)	55/89 (61.8)	0.119
- Non-hospitalised	7/27 (25.9)	34/66 (51.5)	47/82 (57.3)	46/89 (51.7)	0.044
- Hospitalised	4/27 (14.8)	1/66 (1.5)	10/82 (12.2)	13/89 (14.6)	0.046
Life-threatening	1/27 (3.7)	0/66 (0.0)	1/82 (1.2)	3/89 (3.4)	0.392

Figures represent medians (25^{th}-75^{th} centiles) and n/N (%).

Clusters were compared using the Kruskal-Wallis/Chi-squared test.

*Pairwise comparisons of the group demonstrated that cluster 1 had a lower exacerbation rate than the other clusters (p=0.016 for cluster 1 vs cluster 2, p=0.003 for cluster 1 vs cluster 3 and p=0.01 for cluster 1 vs cluster 4).

**Pairwise comparisons of the group and review of the mean exacerbation rate for each cluster, demonstrated that cluster 2 had a lower exacerbation rate than the other clusters (p=0.013 for cluster 1 vs cluster 2, p=0.016 for cluster 2 vs cluster 3, p=0.005 for cluster 2 vs cluster 4).

Table 44 Details of Prospective Exacerbations in the Adult ISAC Component Atopy Clusters

	Non-sensitised	ISAC Cluster 1	ISAC Cluster 2	ISAC Cluster 3	ISAC Cluster 4	p-value
	n=158	n=20	n=28	n=28	n=59	
Exacerbation rate (per year):						
All exacerbations	1.7 (0-3)	1.5 (0-3)	1 (0-3.5)	1 (0-3.5)	1 (0-2)	0.041*
Moderate exacerbations	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.276
Severe exacerbations	1 (0-2.8)	1 (0-2.5)	1.2 (0-3.5)	1 (0-2.5)	0 (0-1)	0.062
- Non-hospitalised	1 (0-2)	1 (0-2)	0 (1-3)	0 (0-1)	0 (0-1)	0.028**
- Hospitalised	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0.5)	0 (0-0)	0.026***
Life-threatening	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	0.128
Any exacerbations (yes/no):						
All exacerbations	117/158 (74.1)	14/20 (70.0)	20/28 (71.4)	16/28 (57.1)	30/59 (50.9)	0.017
Moderate exacerbations	27/158 (17.1)	1/20 (5.0)	3/28 (10.7)	2/28 (7.1)	5/59 (8.5)	0.238
Severe exacerbations	99/158 (62.7)	14/20 (70.0)	17/28 (60.7)	15/28 (53.6)	24/59 (40.7)	0.038
- Non-hospitalised	89/158 (56.3)	13/20 (65.0)	16/28 (57.1)	9/28 (32.1)	23/59 (39.0)	0.025
- Hospitalised	13/158 (8.2)	2/20 (10.0)	6/28 (21.4)	7/28 (25.0)	5/59 (8.5)	0.038
Life-threatening	1/158 (0.6)	1/20 (5.0)	0/28 (0.0)	2/28 (7.1)	2/59 (3.4)	0.126

Figures represent medians (25th-75th centiles) and n/N (%).

Clusters were compared using the Kruskal-Wallis/Chi-squared test.

ISAC Cluster 1= Multiple sensitisation; ISAC Cluster 2= House dust mite sensitisation; ISAC Cluster 3= Grass pollen sensitisation; ISAC Cluster 4= Miscellaneous sensitisation.

*Pairwise comparisons of the group demonstrated that cluster 4 had a lower exacerbation rate than the non-sensitised cluster (p=0.002) and cluster 2 (p=0.033).

**Pairwise comparisons of the group demonstrated that the non-sensitised cluster had a higher exacerbation rate than cluster 3 (p=0.026) and cluster 4 (p=0.022), cluster 1 had a higher exacerbation rate than cluster 3 (p=0.033) and cluster 4 (p=0.046) and cluster 2 had a higher exacerbation rate than cluster 3 (p=0.048).

***Pairwise comparison of the group demonstrated that cluster 3 had a higher exacerbation rate than the non-sensitised cluster (p=0.005) and cluster 4 (p=0.021) and the non-sensitised cluster had a higher exacerbation rate than cluster 2 (p=0.049).

6.3 Risk Factors for Severe Exacerbations

6.3.1 Paediatric Participants- Univariate Analysis

In univariate analysis, female gender (IRR 1.94, 95% CI 1.20-1.32, p=0.007), increasing BMI (IRR 1.23, 95% CI 1.03-1.47, p=0.020), a higher number of exacerbations in the previous year (IRR 1.20, 95% CI 1.09-1.32, p <0.001), eczema (IRR 2.27, 95% CI 1.29-4.01, p=0.005) and miscellaneous allergen sensitisation (IRR 2.09, 95% 1.05-4.14, p=0.035) were associated with an increased risk of future severe asthma exacerbations when preschool children and school aged children were analysed together (Table 45). Participants who reported pets (IRR 1.78, 95% CI 1.05-3.00, p=0.032), air pollutants (IRR 1.95, 95% CI 1.12-3.38, p=0.018) and stress (IRR 2.24, 95% CI 1.33-3.78, p=0.002) as symptom triggers were also more likely to experience future exacerbations. A higher FEV_1% predicted (IRR 0.98, 95% CI 0.97-0.99, p=0.005), a higher asthma control z-score (IRR 0.53, 95% CI 0.39-0.72, p<0.001) and a higher quality of life z-score (IRR 0.68, 95% CI 0.51-0.89, p=0.006) were associated with a lower risk of future severe exacerbations.

When school aged children were analysed independently, the only factors associated with future severe exacerbations were female gender (IRR 3.32, 95% CI 1.85, p <0.001), increasing weight (IRR 1.29, 95% CI 1.00-1.65, p=0.048), a higher number of exacerbations in the previous year (IRR 1.19, 95% CI 1.05-1.33, p=0.005) and better medication adherence (IRR 1.19, 95% CI 1.00-1.41, p=0.049) (Table 45). Once again, a higher FEV_1% predicted (IRR 0.98, 95% CI 0.97-1.00, p=0.010), a higher asthma control z-score (IRR 0.49, 95% CI 0.35-0.68, p<0.001) and a higher quality of life z-score (IRR 0.69, 95% CI 0.49-0.95, p=0.025) were associated with a lower risk of future severe exacerbations.

For preschool children, a higher number of exacerbations in the previous year (IRR 1.21, 95% CI 1.04-1.40, p=0.015), eczema (IRR 2.36, 95% CI 1.09-5.59, p=0.031) and stress as a symptom trigger (IRR 3.08, 95% CI 1.32-7.15, p=0.009) were associated with future severe exacerbations. A higher quality of life z-score was associated with fewer exacerbations in the preceding year (IRR 0.53, 95% CI 0.32-.0.89, p=0.017).

Table 45 Association between Specific Clinical Characteristics and Future Severe Exacerbations in Paediatric Participants- Univariate Analysis

	All Participants with Severe Asthma/ Wheeze	School Aged Children with Severe Asthma (SA)	Preschool Children with Severe Wheeze (SW)
Demographic details			
Female	1.94 [1.20-3.12] (0.007)	3.32 [1.85-5.95] (<0.001)	0.63 [0.28-1.43] (0.270)
Age (years)	1.04 [0.99-1.10] (0.083)	1.00 [0.89-1.12] (0.976)	0.95 [0.66-1.35] (0.766)
Caucasian	0.82 [0.46-1.46] (0.500)	1.00 [0.49-2.05] (0.990)	0.60 [0.24-1.51] (0.277)
Anthropometry			
Height z-score	0.88 [0.71-1.09] (0.250)	1.06 [0.78-1.45] (0.688)	0.80 [0.59-1.08] (0.140)
Weight z-score	1.17 [0.95-1.42] (0.134)	1.29 [1.00-1.65] (0.048)	0.93 [0.66-1.30] (0.672)
BMI z-score	1.23 [1.03-1.47] (0.020)	1.19 [0.95-1.48] (0.123)	1.20 [0.86-1.68] (0.287)
Asthma/wheeze history			
Age at diagnosis (years)	0.96 [0.85-1.10] (0.552)	0.92 [0.81-1.05] (0.203)	0.98 [0.57-1.68[(0.946)
ICU admission ever	1.15 [0.58-2.28] (0.684)	1.00 [0.45-2.21] (0.996)	1.26 [0.35-4.55] (0.720)
ICU admission in past year	1.01 [0.35-2.95] (0.983)	1.17 [0.24-5.71] (0.845)	1.06 [0.25-4.44] (0.941)
Number of exacerbations in previous year	1.20 [1.09-1.32] (<0.001)	1.19 [1.05-1.33] (0.005)	1.21 [1.04-1.40] (0.015)
Reported triggers for respiratory symptoms			
Respiratory infections	7.87 [0.65-94.66] (0.104)	9.69 [0.82-114.03] (0.071)	-
Pets	1.78 [1.05-3.00] (0.032)	1.39 [0.69-2.80] (0.356)	1.73 [0.58-5.16] (0.324)
Exercise	1.72 [0.80-3.70] (0.162)	0.97 [0.31-3.09] (0.965)	2.38 [0.81-6.97] (0.113)
Cold air	1.68 [0.81-3.48] (0.165)	2.05 [0.82-5.15] (0.127)	1.14 [0.36-3.61] (0.819)
Air pollutants	1.95 [1.12-3.38] (0.018)	1.43 [0.70-2.94] (0.326)	1.94 [0.81-4.67] (0.138)
Stress	2.24 [1.33-3.78] (0.002)	1.67 [0.87-3.23] (0.124)	3.08 [1.32-7.15] (0.009)
Pollens	1.71 [0.95-3.07] (0.073)	1.33 [0.57-3.12] (0.515)	1.64 [0.65-4.15] (0.298)
Other medical problems			
Diagnosed hay fever	1.37 [0.79-2.38] (0.258)	1.27 [0.54-3.02] (0.581)	0.91 [0.38-2.14] (0.831)
Diagnosed allergic rhinitis	1.18 [0.71-1.97] (0.515)	1.01 [0.53-1.94] (0.975)	0.89 [0.35-2.26] (0.808)
Diagnosed eczema	2.27 [1.29-4.01] (0.005)	1.80 [0.81-4.02] (0.151)	2.46 [1.09-5.59] (0.031)
Food allergy	1.12 [0.61-2.06] (0.703)	0.98 [0.48-1.97] (0.949)	1.05 [0.31-3.57] (0.932)
Allergic sensitisation			
Grass pollen	1.24 [0.76-2.01] (0.381)	0.97 [0.51-1.84] (0.932)	1.16 [0.38-3.51] (0.794)
Tree pollen	1.25 [0.75-2.09] (0.389)	1.00 [0.53-1.88] (0.996)	1.07 [0.33-3.50] (0.906)
Dog	1.43 [0.88-2.33] (0.148)	1.21 [0.64-2.30] (0.562)	1.31 [0.46-3.74] (0.614)
Cat	1.47 [0.90-2.38] (0.123)	1.19 [0.61-2.30] (0.609)	1.53 [0.61-3.83] (0.360)
House dust mite	1.07 [0.66-1.75] (0.770)	0.83 [0.44-1.57] (0.567)	1.21 [0.54-2.70] (0.640)
Mould	1.28 [0.75-2.18] (0.367)	1.06 [0.56-2.01) [0.857]	1.15 [0.28-4.64] (0.849)
Atopy	1.43 [0.81-2.50] (0.217)	0.92 [0.35-2.41] (0.859)	1.55 [0.71-3.35] (0.270)

	All Participants with Severe Asthma/ Wheeze	School Aged Children with Severe Asthma (SA)	Preschool Children with Severe Wheeze (SW)
Pet exposure and sensitisation			
Dog (not exposed, not sensitised vs) *			
Exposed, not sensitised	0.99 [0.43-2.25] (0.976)	1.53 [0.51-4.61] (0.448)	0.26 [0.05-1.35] (0.110)
Not exposed, sensitised	1.44 [0.83-2.47] (0.193)	1.38 [(0.64-2.95] (0.411)	1.34 [0.48-3.73] (0.580)
Exposed and sensitised	1.41 [0.58-3.39] (0.448)	1.46 [0.52-4.08] (0.475)	-
Cat (not exposed, not sensitised vs)*			
Exposed, not sensitised	0.84 [0.31-2.29] (0.735)	2.82 [0.49-16.43] (0.248)	0.30 [0.07-1.30] (0.108)
Not exposed, sensitised	1.41 [0.83-2.40] (0.203)	1.28 [0.64-2.59] (0.488)	1.56 [0.61-3.97] (0.348)
Exposed and sensitised	1.55 [0.63-3.80] (0.343)	1.67 [0.58-4.77] (0.343)	0.50 [0.05-5.27] (0.563)
Second-hand smoke exposure	0.73 [0.37-1.43] (0.357)	0.65 [0.29-1.46] (0.297)	0.80 [0.24-2.63] (0.715)
Spirometry			
FEV_1 % predicted	0.98 [0.97-0.99] (0.005)	0.98 [0.97-1.00] (0.010)	0.97 [0.92-1.02] (0.255)
FEV_1/FVC ratio	0.24 [0.02-2.77] (0.255)	0.26 [0.01-4.77] (0.361)	0.06 [0.00-192.01] (0.501)
Quality of life and asthma control			
Asthma control z-score	0.53 [0.39-0.72] (<0.001)	0.49 [0.35-0.68] (<0.001)	0.63 [0.34-1.66] (0.143)
Quality of life z-score	0.68 [0.51-0.89] (0.006)	0.69 [0.49-0.95] (0.025)	0.53 [0.32-0.89] (0.017)
MARS total score	1.05 [0.94-1.17] (0.422)	1.19 [1.00-1.41] (0.049)	0.94 [0.82-1.09] (0.411)
Clinical cluster allocation (cluster 3 vs)			
Cluster 1	1.67 [0.65-4.34] (0.289)	1.82 [0.58-5.72] (0.303)	1.49 [0.35-6.31] (0.587)
Cluster 2	0.87 [0.33-2.24] (0.767)	0.96 [0.29-3.18] (0.949)	0.76 [0.19-3.01] (0.699)
Cluster 4	1.05 [0.53-2.08] (0.897)	1.33 [0.55-3.17] (0.526)	0.74 [0.28-1.97] (0.546)
Cluster 5	1.47 [0.70-3.08] (0.310)	2.07 [0.85-5.01] (0.108)	0.44 [0.11-1.76] (0.244)
Cluster 6	1.02 [0.28-3.77] (0.976)	3.21 [0.55-18.72] (0.196)	-
ISAC component atopy cluster allocation (not sensitised vs)			
Cluster 1 (Multiple sensitisation)	1.08 [0.51-2.28] (0.835)	0.67 [0.24-1.82] (0.430)	1.33 [0.41-4.35] (0.638)
Cluster 2 (House mite sensitisation)	0.98 [0.50-1.92] (0.953)	0.52 [0.20-1.34] (0.176)	1.66 [0.61-4.53] (0.324)
Cluster 3 (Grass pollen sensitisation)	1.42 [0.48-4.23] (0.527)	0.85 [0.25-2.88] (0.793)	-
Cluster 4 (Miscellaneous sensitisation)	2.09 [1.05-4.14] (0.035)	1.25 [0.52-3.01] (0.621)	-

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).

Sensitisation was defined as a specific IgE level ≥ 0.35 kU/l or a wheal ≥3mm on skin prick testing.

Atopy was defined as sensitisation to one or more of the following aeroallergens: tree pollen, grass pollen, cat, dog, house dust mite and mould.

Food allergy was defined as a history of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with a food plus a positive skin prick test (≥3mm wheal) or positive specific IgE (≥ 0.35 kU/l) to that food.

*Exposure refers to the presence of a dog/cat at home.

A higher asthma control z-score is associated with better asthma control.

6.3.2 Paediatric Participants- Multivariable Analysis

In multivariable analysis of school aged participants, female gender (IRR 3.17, 95% CI 1.78-5.64, p<0.001), increasing weight (IRR 1.27, 95% CI 1.03-1.56, p=0.023), a higher number of exacerbations in the previous year (IRR 1.18, 95% CI 1.07-1.31, p=0.002) and a higher quality of life (IRR 1.56, 95% CI 1.07-2.79, p=0.022) were associated with an increased risk of future severe exacerbations. Better asthma control was associated with a lower risk of future severe asthma exacerbations (IRR 0.44, 95% CI 0.29-0.67, p=0.002) (Table 46).

For preschool children, stress as a symptom trigger was associated with an increased risk of future severe exacerbations (IRR 3.17, 95% 1.37-7.37, p=0.007). Similar to school aged children, a higher number of exacerbations in the previous year was also associated with an increased risk of future severe exacerbations (IRR 1.18, 95% CI 1.02-1.37, p=0.031) (Table 47).

In combined analysis of preschool and school aged children, female gender (IRR 1.79, 95% CI 1.11-2.91, p=0.018) and a higher number of exacerbations in the previous year (IRR 1.13, 95% CI 1.03-1.24, p=0.007) were associated with an increased risk of future severe exacerbations. Better asthma control was associated with a lower risk of future exacerbations (IRR 0.57, 0.43-0.76, p<0.001). Age category did not influence the risk of future severe exacerbations (Table 48). None of the clinical clusters were associated with an increased risk of future severe asthma exacerbations (Table 49). Compared to non-sensitised individuals, children in ISAC component atopy cluster 4 with miscellaneous sensitisation were more likely to experience future severe exacerbations (IRR 2.01, 95% CI 1.06-3.81, p=0.034) (Table 49).

Table 46 Association between Specific Clinical Characteristics and Future Severe Exacerbations in School Aged Children with Severe Asthma- Multivariable Analysis

	Univariate analysis	Multivariable analysis
Female	3.32 [1.85-5.95] (<0.001)	3.17 [1.78-5.64] (<0.001)
Weight z-score	1.29 [1.00-1.65] (0.048	1.27 [1.03-1.56] (0.023)
Number of exacerbations in the previous year	1.19 [1.05-1.33] (0.005)	1.18 [1.07-1.31] (0.002)
Asthma control z-score	0.49 [0.35-0.68] (<0.001)	0.44 [0.29-0.67] (0.002)
Quality of life z-score	0.69 [0.49-0.95] (0.025)	1.56 [1.07-2.29] (0.022)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).
Variables with a p-value <0.1 in univariate analysis were entered into the model and backward deletion was applied until only variables with a p-value <0.05 remained.
A higher asthma control z-score is associated with better asthma control.
A higher quality of life z-score is associated with better quality of life.

Table 47 Association between Specific Clinical Characteristics and Future Severe Exacerbations in Preschool Children with Severe Wheeze- Multivariable Analysis

	Univariate analysis	Multivariable analysis
Number of exacerbations in the previous year	1.21 [1.04-1.40] (0.015)	1.18 [1.02-1.37] (0.031)
Symptoms triggered by stress	3.08 [1.32-7.15] (0.009)	3.17 [1.37-7.37] (0.007)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).
Variables with a p-value <0.1 in univariate analysis were entered into the model and backward deletion was applied until only variables with a p-value <0.05 remained.

Table 48 Combined Multivariable Model for all Paediatric Participants

	Univariate analysis	Multivariable analysis
Female	1.94 [1.20-3.12] (0.007)	1.79 [1.11-2.91] (0.018)
Number of exacerbations in the previous year	1.20 [1.09-1.32] (<0.001)	1.13 [1.03-1.24] (0.007)
Asthma control z-score	0.53 [0.39-0.72] (<0.001)	0.57 [0.43-0.76] (<0.001)
School aged (vs preschool age)	1.71 [1.05-2.80] (0.031)	1.40 [0.80-2.45] (0.238)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).
Variables significant in the individual models for preschool and school aged children were included in this model. Backward deletion was applied until only variables with a p-value <0.05 remained. Age group was also included.

Table 49 Multivariable Models to assess the Association between Cluster Assignment and Future Severe Exacerbations in Paediatric Participants

	Multivariable analysis	**With ISAC clusters**	**With clinical clusters**
Female	1.94 [1.20-3.12] (0.007)	1.73 [1.07-2.80] (0.025)	1.82 [1.09-3.05] (0.022)
Number of exacerbations in the previous year	1.20 [1.09-1.32] (<0.001)	1.17 [1.07-1.27] (<0.001)	1.13 [1.02-1.26] (0.019)
Asthma control z-score	0.53 [0.39-0.72] (<0.001)	0.51 [0.39-0.69] (<0.001)	0.59 [0.43-0.80] (0.001)
School aged (vs preschool age)	1.71 [1.05-2.80] (0.031)	0.99 [0.54-1.80] (0.974)	1.41 [0.78-2.52] (0.252)
ISAC component atopy cluster allocation (not sensitised vs)			
Cluster 1 (Multiple sensitisation)		0.60 [0.29-1.25] (0.172)	
Cluster 2 (House mite sensitisation)		1.23 [0.64-2.34] (0.535)	
Cluster 3 (Grass pollen sensitisation)		1.41 [0.54-3.65] (0.483)	
Cluster 4 (Miscellaneous sensitisation)		2.01 [1.06-3.81] (0.034)	
Clinical cluster allocation (cluster 3 vs)			
Cluster 1			1.25 [0.48-3.19] (0.651)
Cluster 2			0.77 [0.27-2.26] (0.640)
Cluster 4			0.95 [0.48-1.89] (0.889)
Cluster 5			1.17 [0.58-2.36] (0.668)
Cluster 6			1.08 [0.29-3.99] (0.907)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).
A higher asthma control z-score is associated with better asthma control.

6.3.3 Adult Participants- Univariate Analysis

In adults with severe asthma, a previous ICU admission (IRR 1.71, 95% CI 1.21-2.42, p=0.002), a higher number of exacerbations in the previous year (IRR 1.21, 95% CI 1.14-1.28, p <0.001) and a higher asthma control z-score were associated with an increased risk of future severe exacerbations (IRR 1.57, 95% CI 1.33-1.87, p <0.001) (Table 50). The following symptom triggers were also associated with a higher risk of future severe exacerbations: respiratory infections (IRR 2.57, 95% CI 1.42-2.64, p=0.002), pets (IRR 1.78, 95% CI 1.30-2.44, p <0.001), cold air (IRR 1.79, 95% CI 1.25-2.57, p=0.002), air pollutants (IRR 1.99, 95% CI 1.38-2.86, p <0.001), stress (IRR 1.40, 95% CI 1.01-1.93, p=0.041) (Table 50). Factors associated with a lower rate of severe exacerbations in the preceding year included older age (IRR 0.99, 95% CI 0.98-1.00, p=0.037), tree pollen sensitisation (IRR 0.59, 95% CI 0.39-0.89, p=0.011) and a higher quality of life (IRR 0.77, 95% CI 0.65-0.92, p=0.004) (Table 50). Participants in clinical cluster 1 (mostly atopic patients with well-controlled asthma, normal lung function and no oral corticosteroid use) were less likely to experience exacerbations than participants in clinical cluster 4 (obese female patients with severe, uncontrolled asthma but normal lung function) (IRR 0.41, 95% CI 0.22-0.78, p=0.006) (Table 50). Regarding atopic sensitisation, patients in cluster 4 with miscellaneous sensitisation had a lower risk of future severe exacerbations compared to non-sensitised individuals (IRR 0.61, 95% CI 0.40-0.92, p=0.020) (Table 50).

Table 50 Association between Specific Clinical Characteristics and Future Severe Exacerbations in Adult Participants- Univariate Analysis

	All adult participants with severe asthma
Demographic details	
Female	1.32 [0.97-1.81] (0.080)
Age (years)	0.99 [0.98-1.00] (0.037)
Caucasian	0.96 [0.55-1.68] (0.898)
Anthropometry	
BMI (kg/m^2)	1.00 [0.97-1.03] (0.938)
Asthma history	
Age at diagnosis (years)	0.99 [0.98-1.00] (0.062)
ICU admission ever	1.71 [1.21-2.42] (0.002)
ICU admission in past year	1.98 [0.92-4.28] (0.083)
Number of exacerbations in previous year	1.21 [1.14-1.28] (<0.001)
Reported triggers for respiratory symptoms	
Respiratory infections	2.57 [1.42-4.64] (0.002)
Pets	1.78 [1.30-2.44] (<0.001)
Exercise	1.54 [0.98-2.41] (0.061)
Cold air	1.79 [1.25-2.57] (0.002)
Air pollutants	1.99 [1.38-2.86] (<0.001)
Stress	1.40 [1.01-1.93] (0.041)
Pollens	1.42 [1.03-1.94] (0.030)
Other medical problems	
Diagnosed hay fever	1.20 [0.87-1.65] (0.260)
Diagnosed allergic rhinitis	0.89 [0.65-1.22] (0.470)
Diagnosed eczema	1.17 [0.84-1.63] (0.345)
Food allergy	0.82 [0.41-1.66] (0.581)
Allergic sensitisation	
Grass pollen	1.09 [0.78-1.51] (0.611)
Tree pollen	0.59 [0.39-0.89] (0.011)
Dog	0.93 [0.67-1.31] (0.685)
Cat	1.02 [0.73-1.43] (0.903)
House dust mite	0.87 [0.63-1.21] (0.416)
Mould	0.85 [0.58-1.25] (0.413)
Atopy	0.77 [0.53-1.12] (0.166]
Pet exposure and sensitisation	
Dog (not exposed, not sensitised vs)*	
Exposed, not sensitised	1.20 [0.73-1.96] (0.474)
Not exposed, sensitised	0.98 [0.67-1.46] (0.959)
Exposed and sensitised	0.90 [0.50-1.61] (0.720)

	All adult participants with severe asthma
Cat (not exposed, not sensitised vs)	
Exposed, not sensitised	0.93 [0.51-1.67] (0.797)
Not exposed, sensitised	1.03 [0.71-1.49] (0.877)
Exposed and sensitised	0.90 [0.47-1.70] (0.743)
Smoking status (non-smoker vs)	
Ex-smoker	1.10 [0.77-1.57] (0.588)
Current smoker	0.89 [0.53-1.57] (0.660)
Spirometry	
FEV_1 % predicted	1.00 [0.99-1.00] (0.379)
FEV_1/FVC ratio	1.36 [0.47-3.95] (0.576)
Asthma control, quality of life and medication adherence	
Asthma control z-score	1.57 [1.33-1.87] (<0.001)
Quality of life z-score	0.77 [0.65-0.92] (0.004)
MARS total score	0.96 [0.90-1.03] (0.307)
Clinical cluster allocation (cluster 4 vs)	
Cluster 1	0.41 [0.22-0.78] (0.006)
Cluster 2	0.72 [0.47-1.09] (0.123)
Cluster 3	0.77 [0.52-1.14] (0.189)
ISAC component atopy cluster allocation (not sensitised vs)	
Cluster 1 (Multiple sensitisation)	0.92 [0.50-1.70] (0.818)
Cluster 2 (House dust mite sensitisation)	1.21 [0.73-2.01] (0.461)
Cluster 3 (Grass pollen sensitisation)	1.16 [0.70-1.93] (0.566)
Cluster 4 (Miscellaneous sensitisation)	0.61 [0.40-0.92] (0.020)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value)

Sensitisation was defined as a specific IgE level ≥ 0.35 kU/l or a wheal ≥3mm on skin prick testing.

Atopy was defined as sensitisation to one or more of the following aeroallergens: tree pollen, grass pollen, cat, dog, house dust mite and mould.

Food allergy was defined as a history of urticaria, angioedema, pruritus, throat tightness, stridor, chest tightness or wheeze within two hours of contact with a food plus a positive skin prick test (≥3mm wheal) or positive specific IgE (≥ 0.35 kU/l) to that food.

*Exposure refers to the presence of a dog/cat at home.

A higher asthma control z-score is associated worse asthma control.

A higher quality of life z-score with a better quality of life.

6.3.4 Adult Participants- Multivariable Analysis

In multivariable analysis, a previous ICU admission (IRR 1.37, 95% CI 1.01-1.86, p=0.045), a higher number of exacerbations in the previous year (IRR 1.18, 95% CI 1.12-1.25, p <0.001), symptoms triggered by pets (IRR 1.43, 95% CI 1.08-1.89, p=0.013) and poor asthma control at baseline (IRR 1.37, 95% CI 1.16-1.61, p <0.001) were identified as risk factors for future severe asthma exacerbations (Table 51). These factors remained significant when the clinical and ISAC component atopy clusters were included in multivariable analysis (Table 52). Adults with miscellaneous sensitisation had a lower risk of future severe exacerbations compared to non-sensitised individuals (IRR 0.57, 95% CI 0.39-0.84, p=0.005) (Table 52). None of the clinical clusters were associated with an increased risk of future exacerbations.

Table 51 Association between Specific Clinical Characteristics and Future Severe Exacerbations in Adult Participants- Multivariable Analysis

	Univariate Analysis	Multivariable Analysis
ICU admission ever	1.71 [1.21-2.42] (0.002)	1.37 [1.01-1.86] (0.045)
Number of exacerbations in previous year	1.21 [1.14-1.28] (<0.001)	1.18 [1.12-1.25] (<0.001)
Symptoms triggered by pets	1.78 [1.30-2.44] (<0.001)	1.43 [1.08-1.89] (0.013)
Asthma control z-score	1.57 [1.33-1.87] (<0.001)	1.37 [1.16-1.61] (<0.001)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).

Factors with a p-value <0.1 in univariate analysis were entered into the model and backward deletion was applied until only factors with a p-value <0.05 remained. Clinical and ISAC component atopy clusters were excluded.

A higher asthma control z-score is associated with worse asthma control.

Table 52 Multivariable Models to assess the Association between Cluster Assignment and Future Severe Exacerbations in Adult Participants

	Multivariable Analysis	**With ISAC clusters**	**With Clinical clusters**
ICU admission ever	1.37 [1.01-1.86] (0.045)	1.44 [1.06-1.95] (0.018)	1.43 [1.04-1.98] (0.026)
Number of exacerbations in the previous year	1.18 [1.12-1.25] (<0.001)	1.17 [1.11-1.23] (<0.001)	1.20 [1.12-1.27] (<0.001)
Symptoms triggered by pets	1.43 [1.08-1.89] (0.013)	1.48 [1.09-2.01] (0.012)	1.58 [1.17-2.14] (0.003)
Asthma control z-score	1.37 [1.16-1.61] (<0.001)	1.38 [1.18-1.62] (<0.001)	1.39 [1.17-1.66] (<0.001)
ISAC component atopy cluster allocation (not sensitised vs)			
Cluster 1 (Multiple sensitisation)		0.87 [0.50-1.50] (0.613)	
Cluster 2 (House dust mite sensitisation)		0.89 [0.54-1.47] (0.643)	
Cluster 3 (Grass pollen sensitisation)		0.71 [0.45-1.13] (0.149)	
Cluster 4 (Miscellaneous sensitisation)		0.57 [0.39-0.84] (0.005)	
Clinical cluster allocation (cluster 4 vs)			
Cluster 1			1.08 [0.59-1.98] (0.798)
Cluster 2			1.19 [0.80-1.76] (0.396)
Cluster 3			0.96 [0.67-1.38] (0.845)

Figures represent incidence rate ratio, IRR [95% confidence intervals] (p-value).
A higher asthma control z-score is associated with worse asthma control.

Chapter 7: Discussion and Conclusions

Wheeze and asthma are major health problems worldwide, affecting all age groups. Preschool children with wheeze have higher morbidity than older children and adults with asthma and consume a disproportionately high amount of health care resources.[9] For similar reasons, severe asthma and asthma exacerbations are also research priorities. This thesis has used data collected as part of the EuroPrevall birth cohort study and Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (UBIOPRED) study to provide new insights into wheeze and asthma across the life course. Areas explored include risk factors for early childhood wheeze, the relationship between allergic manifestations and asthma/wheeze severity and risk factors for exacerbations in patients with severe asthma/preschool wheeze.

7.1 Early Childhood Wheeze across Europe

Early childhood wheeze is highly prevalent affecting up to 50% of children in the first six years of life.[2] Although several genetic and environmental risk factors have been identified, an improved understanding of the aetiology of early childhood wheeze is required to enable the development of preventative strategies. Comparing disease prevalence between populations may provide aetiological clues.[22] Two major studies (ISAAC[152] and the ECRHS[23]) have demonstrated that there are large geographical differences in asthma prevalence in school aged children and adults, respectively. One previous study has compared prevalence rates of asthma and wheeze at age 4 across Europe (Uphoff et al.). However, this study utilised data from ten independent cohorts in eight countries.[29] This is the first study to examine international variations in the prevalence of preschool wheeze within a single multi-centre cohort.

7.1.1 Prevalence Estimates of Wheeze

This thesis has demonstrated that the prevalence of parent-reported wheeze in the first two years of life varies considerably across Europe with a broadly north-western to south-eastern gradient. The prevalence of wheeze in the second year of life across all nine study centres was 7.8%, ranging from ≤3% in Lodz (Poland), Vilnius (Lithuania), Athens (Greece), and Madrid (Spain) to 11.8% in Berlin (Germany), 13.1% in Southampton (UK) and 17.2 % in Reykjavik (Iceland). The prevalence of recurrent wheeze (wheeze in the first and second years of life) was also highest in Reykjavik (10%) and Southampton (7.9%) and lowest in Lodz, Vilnius and Athens (≤1%).

Other birth cohorts in which the prevalence of wheeze at 2 years of age has been reported include the PARIS (2003-2006), PIAMA (1996-1997) and Generation R cohorts (2002-2006). 3840

children were enrolled in the PARIS birth cohort and were followed up regularly from birth to 4 years. Amongst the 1773 children with complete follow up data, the prevalence of wheeze between the ages of 1-2 years was 12%.[40] This is similar to estimates of 13% for Southampton, 12% for Berlin and 11% for Amsterdam in the EuroPrevall cohort. Within the Dutch-based Generation R and PIAMA cohorts the prevalence of parent reported wheeze at 2 years of age was, however, higher at 20.1% [45] and 18%, [13] respectively. In all of these studies, questions relating to wheeze were adapted from ISAAC. In the PARIS, PIAMA and Generation R studies questionnaires were self-administered whereas in EuroPrevall they were conducted over the phone by trained personnel.

As previously mentioned, data from ten birth cohorts, including the PARIS and PIAMA cohorts, was utilised by Uphoff et al. to examine variations in prevalence rates of asthma and wheeze at 4 years in Europe. Countries represented included Sweden (BAMSE), England (BiB), Germany (GINIplus, MAS, and LISAplus), Spain (INMA), France (PARIS), The Netherlands (PIAMA), Greece (RHEA) and Italy (ROBBIC). Prevalence rates of wheeze ever at 4 years were lowest in Greece (9.8%) and France (15.1%) and highest in Germany (42.8%) and Spain (55.4%). High levels of wheeze in Spain contrast with the findings of this thesis. However, the INMA cohort was reportedly not representative of the general population as families were mainly recruited from urban areas. As acknowledged by Uphoff et al., comparing data from independent cohorts has limitations due to use of different inclusion and exclusion criteria and variations in time periods of collection. Furthermore, for some cohorts outcome variables were generated from data collected at different time points, leading to overestimation.[29] The conclusions drawn by this thesis on how prevalence rates of preschool wheeze differ across Europe are therefore likely to be more accurate.

When the findings of this thesis were compared with those of ISAAC and the ECHRS, similarities were observed. ISAAC, which commenced in 1991, comprised three phases. Phase one used standardised questionnaires to describe the prevalence of wheeze, asthma, eczema and allergic rhinitis in 463,801 children aged 13-14 years from 56 countries and 257,800 children aged 6-7 years from 38 countries. Phase two subsequently investigated potential aetiological factors contributing to the international differences observed in phase one, whilst phase three was a repetition of phase one to allow time trends in prevalence to be assessed. The ECHRS was established in 1988. 138,565 adults aged 20-44 years from 48 centres across 22 countries completed a phase one screening questionnaire on asthma symptoms and medication use. A subsample of participants were subsequently studied in more detail in phase two.[28] Questions used in ISAAC and the ECHRS included 'Have you had wheezing or whistling in the chest in the last 12 months?' and 'Have you ever had asthma?' In keeping with the findings of this thesis, the

ISAAC study and ECRHS reported a high prevalence of wheeze in Western Europe with lower prevalences in Eastern and Southern Europe.[28] Five countries were represented in EuroPrevall, ISAAC and ECRHS: the UK, Germany, Italy, Spain and Greece. In EuroPrevall and ISAAC (13-14 year olds), the prevalence of wheeze in the last 12 months was highest in the UK and lowest in Greece. In the ECHRS, the UK had the highest prevalence of wheeze in the last 12 months whilst Italy had the lowest and Greece the second lowest. The rankings of asthma prevalence in ISAAC and ECHRS were also similar to those of wheeze in EuroPrevall. This is not surprising given that early childhood wheeze is a risk factor for asthma in later life. The prevalence of wheeze was higher in ISAAC and the ECHRS than in EuroPrevall. For example, in the UK the prevalence of wheeze in the last 12 months was 13.1% in EuroPrevall compared to 32.2% at age 13-14 years in ISAAC and 25.2-29.8% in ECHRS. There are many potential reasons for this. Firstly, wheeze is a symptom with numerous aetiologies, which differ in children and adults. Secondly, not all older children/adults with asthma experience early childhood wheeze and vice versa. Stern et al., for example, demonstrated that 25.8% of young adults with asthma, never experienced childhood wheeze and of children with wheeze, 51.8% do not develop asthma.[153] Thirdly, there may be a cohort effect related to the fact that participants in ECHRS were born up to 50 years before those in EuroPrevall and hence were exposed to different environmental influences in early life. Another noteworthy finding of ISAAC and the ECHRS is that prevalence rates of wheeze and asthma varied both between and within countries.[152] If the same is true for preschool wheeze, this may partly explain why the prevalence of wheeze at 2 years in EuroPrevall differed from that reported by other European birth cohort studies.

In summary, prevalence rates of wheeze in the first two years of life varied considerably between centres in the EuroPrevall birth cohort study with similar prevalence patterns to those observed in older children and adults in the ISAAC and ECHRS.

7.1.2 Risk factors for Early Childhood Wheeze

The second aim of this analysis was to evaluate risk factors for early childhood wheeze and how these differ across Europe, focusing on food allergy, infant feeding and smoke exposure. Other known risk factors for early childhood wheeze, such male gender, a family history of allergic disease, respiratory tract infections and day care attendance were taken into account. Given that risk factors for a disease may differ between populations, risk factors for wheeze were investigated for the EuroPrevall cohort as a whole and by study centre.

7.1.2.1 Food Allergy

The atopic march describes the natural progression from atopic dermatitis (eczema) in early childhood to asthma and allergic rhinitis later in childhood. Although the role of food allergy in this is unclear, food allergy is associated with an increased incidence of asthma at school age[96] and worse asthma outcomes.[92] Few studies have looked at the association between food allergy and early life wheeze. In the Urban Environment and Childhood Asthma (URECA) birth cohort study, food allergy was associated with wheeze in the third, fourth and fifth years of life but there was no association between food allergy and wheeze in the first two years of life. A limitation of the URECA study is that food allergy was defined on the basis of positive IgE levels and a history suggestive of allergy rather than the outcome of double-blind, placebo-controlled food challenges (DBPCFCs). Furthermore, only 3 foods (milk, egg and peanut) were considered and all children were recruited from inner-city areas in the United States.[94] Although the prevalence of wheeze in EuroPrevall participants with food allergy was higher than the prevalence of wheeze in those without food allergy (21.5 vs 7.6%), no association between food allergy and wheeze in the second year of life was found when potential confounders were considered. This remained the case when IgE mediated food allergy was considered separately (in a post hoc analysis). This may be due to the fact that wheeze in the first two years of life is predominantly driven by respiratory tract infections rather than atopy.

7.1.2.2 Infant Feeding Practices

Given that breast milk contains antiviral antibodies, a protective effect on early childhood wheeze is plausible. [49] It has previously been concluded that exclusive breastfeeding for at least 4 months reduces the risk of recurrent wheeze in childhood.[49,50] However, no association between breastfeeding or breastfeeding duration and wheeze in the second year of life was found in the EuroPrevall cohort. Even when the relationship between breastfeeding duration and wheeze was analysed using a categorical variable based on quartiles (in a post hoc analysis), no association was seen. This may be due to the fact that the characteristics of the EuroPrevall cohort differed from those in which breastfeeding has been shown to protect against early childhood wheeze. Increased overlap between breastfeeding and solids showed a small protective effect against wheeze in sensitivity model one. Grimshaw et al. previously demonstrated that concurrent feeding with breast and cow's milk reduces the risk of food allergy in infancy,[154] whilst Snijders et al. reported that delaying the introduction of cow's milk and solids increases the risk of eczema and wheeze, respectively.[57] A potential explanation for these findings is that breast milk only has beneficial immunomodulatory effects when the immune system is exposed to other dietary

proteins.[154] Further research is needed to establish whether maximising the duration of overlap between breast and complementary feeding could help to prevent childhood wheeze.

7.1.2.3 Smoke Exposure

Of the smoking variables evaluated, only maternal smoking at one-year follow up was independently associated with wheeze in the second year of life. This suggests that providing advice to new mothers regarding smoking cessation may help to reduce the burden of early childhood wheeze. Regarding maternal smoking during pregnancy, previous studies have demonstrated there are critical time periods of exposure[30,45-47,155] and that the risk of preschool wheeze and asthma increases in a dose-dependent manner.[44] In this analysis, the timing of smoke exposure and number of cigarettes smoked by mothers during pregnancy was not considered. This may account for the fact that smoking was not identified as a risk factor for wheeze in the EuroPrevall cohort. The results of this analysis do in fact agree with those of the Generation R study, which found that children not exposed to smoking during fetal life only had higher risks of wheezing at ages 3 and 4 but not at ages 1 and 2.[45] An unexpected finding of the EuroPrevall study was that the presence of other household smokers was associated with a lower risk of wheeze in the second year of life in univariate analysis. However, once potential confounders were considered, there was a non-significant trend for other household smokers to increase the risk of wheeze.

7.1.2.4 Respiratory Tract Infections

The prevalence of both upper and lower respiratory tract infections varied considerably across Europe with the highest prevalence in Reykjavik and the lowest prevalence in Vilnius. Lower respiratory tract infections were identified as the strongest risk factor for wheeze in the second year of life by the primary model. In the three sensitivity models, upper respiratory tract infections were also associated with wheeze. An association between respiratory tract infections and wheeze is supported by previous research.[9] For example, among a group of children at increased risk of allergies and asthma, Lemanske et al. found that the most significant risk factor for wheeze in the third year of life was symptomatic rhinovirus illnesses during infancy (OR 6.6, $p<0.0001$). This compares to odds ratio of 2.1 for passive smoke exposure, 2.5 for older siblings and 2.0 for allergic sensitisation to foods at one year of age.[36] Colder temperatures and low humidity increase the occurrence of respiratory tract infections.[156,157] Given that respiratory tract infections are associated with wheeze, climatic differences may therefore partly explain variations in the prevalence of early childhood wheeze across Europe.

In the EuroPrevall study, diagnoses of respiratory tract infections were based on parental report. Parents may not understand the difference between upper and lower respiratory tract infections. Therefore, the fact that lower respiratory tract infections were more strongly associated with wheeze than upper respiratory tract infections may be of limited significance. This finding may also be explained by the fact that wheeze is a symptom of lower respiratory tract infections. The 24-month questionnaire asked parents specifically about wheeze without colds in the past 12 months, whereas the 12-month questionnaire asked about wheeze in general in the past 12 months. A small number of 12-month questionnaires were completed within the time range for two-year data. Therefore, some children with wheeze in the second year of life may have had this at the time of a lower respiratory tract infection. Nevertheless, the findings of this analysis suggest that respiratory tract infections are more important than food allergy and infant feeding practices in the aetiology of early childhood wheeze.

7.1.2.5 Day Care Attendance

Day care attendance increases exposure to respiratory tract infections.[38] Therefore, it is not surprising that this was associated with wheeze in the second year of life. The PARIS and PIAMA birth cohorts also found that early day care attendance is associated with increased wheeze before the age of 4 years.[38,40] Several studies have, however, reported a protective effect of day care attendance on asthma at school age,[39,41,158] reflecting the fact that preschool wheeze and asthma are different entities. A post hoc analysis was undertaken to determine whether the relationship between day care and wheeze was influenced by age at entry to day care or the number of hours spent in day care in the first year of life in the EuroPrevall cohort. In univariate analysis, entering day care later was associated with a lower risk of wheeze (IRR 0.98 (per month increase), p=0.26, 95% CI 0.97-1.00). However, neither age at entry to day care nor the number of hours spent in day care in the first year of life was significantly associated with wheeze in the second year of life when included in the primary model.

7.1.2.6 Eczema

Studies have previously demonstrated that childhood eczema is associated with both wheeze/asthma and food allergy. Within the Tucson Children's Respiratory Study for example, the adjusted odds ratio for recurrent wheeze in those with eczema was 2.4 (95% CI 1.3-4.6) when compared to those without eczema.[2] In the HealthNuts study, (a large, population-based study), 4453 one-year old infants were assessed for a history of eczema and underwent skin prick testing to peanut, egg and sesame. Those with eczema had a significantly increased risk of being allergic to peanut, egg or sesame (OR 6.2, 95% CI 4.9-7.9, p <0.001).[159] In the aforementioned URECA study, children with food allergy were also more likely to have eczema in the first three years of

life (adjusted OR 3.6, 95% CI 1.8-7.1 for eczema in the first year of life).[94] Within the EuroPrevall cohort, the prevalence of wheeze in the second year of life was 12.2% in those with eczema compared to 5.1% in those without eczema. However, eczema was only identified as an independent risk factor for wheeze in the second year of life by sensitivity models one and three and not by the primary model or sensitivity model two. This was also the case for recurrent wheeze (wheeze in both the first and second years of life). Studies that have explored the relationship between eczema and wheeze phenotypes based on the longitudinal trajectory of wheeze have shown that eczema is a stronger risk factor for persistent wheeze than transient wheeze. Kurukulaaratchy et al., for example, found that eczema was more common in persistent wheezers (children with wheeze in the first 4 year of life and at age 10) and late-onset wheezers (children with wheeze from 5 years onwards and at age 10) than non-wheezers (40.3 vs 21.3%, p <0.001 and 40.7 vs 21.3%, p <0.001). There was, however, no difference between transient early wheezers (children with wheeze in the first 4 years of life but not at age 10) and non-wheezers (30.7 vs 21.3%).[15] Lack of an association between eczema and wheeze in the EuroPrevall cohort may therefore be due to the fact that some children in the EuroPrevall cohort will be transient wheezers. It is also possible that some children with viral rashes may have been wrongly classified as having eczema. This is suggested by the fact that the prevalence of eczema in individual centres was largely concordant with the prevalence of upper respiratory tract infections.

7.1.2.7 Familial Allergic Disease

Parental allergy also appears to be a more important risk factor for some wheeze phenotypes than others. Within the Tucson Children's Respiratory Study, for example, maternal asthma was associated with late-onset wheezing (aOR 2.8, 95% CI 1.4-5.5) and persistent wheezing (aOR 4.1, 95% CI 2.1-7.9) but not transient early wheezing (aOR 1.6, 95% CI 0.8-3.2).[2] Similarly, in the (PARIS) birth cohort, a parental history of allergic asthma, eczema and/or allergic rhinitis was not associated with transient wheeze in the first four years of life.[40] In the EuroPrevall cohort neither maternal nor paternal allergy (self-reported, doctor diagnosed asthma, eczema or allergic rhinitis) was associated with wheeze in the second year of life according to the primary multivariable model. Self-reported, doctor diagnosed maternal asthma and any paternal allergy were, however, independently associated with recurrent wheeze (wheeze in the both the first and second years of life). This may be due to the fact that children with wheeze in both the first and second years of life are more likely to have persistent wheeze later in childhood than those with wheeze in just the second year of life.

7.1.3 Strengths and Limitations

A major strength of the EuroPrevall study is its' size and the fact that children were recruited from nine centres in nine different countries across Europe. Across the ten Mechanisms of the Development of ALLergy (MeDALL) cohorts included in Uphoff et al.'s study examining variations in the prevalence of wheeze across Europe, the largest number of children in any cohort was 5591 (GINIplus).[29] This compares to 12,049 children in the EuroPrevall cohort. Gene-environment interactions are the basis for childhood wheeze and asthma.[60] This means that susceptible individuals may develop wheeze/asthma in one environment but not another.[160] Multi-centre studies, in which participants encounter different environmental exposures are therefore preferable to single-centre studies for investigating risk factors. In the EuroPrevall cohort, there were large differences between centres in terms of baseline factors and environmental exposures, making it an ideal cohort in which to evaluate risk factors for early childhood wheeze. As previously discussed, comparing data from independent cohorts such as the MeDALL cohorts has limitations due to methodological differences. The EuroPrevall study therefore offers one of the most accurate insights to date on how prevalence rates of early childhood wheeze vary across Europe. There was, however, only one centre per country in the EuroPrevall study. The ISAAC study and ECHRS demonstrated variation in wheeze/asthma prevalence rates both between and within countries. Therefore, the wheeze prevalence rates reported for each EuroPrevall study centre may not be valid at a country level. Another major strength of the EuroPrevall is that double-blind, placebo controlled food challenges were used to diagnose food allergies. This makes it superior to previous studies investigating the relationship between food allergy and early childhood wheeze.

A potential limitation of any longitudinal study is loss to follow up. In this analysis, two-year follow up data were available in 70% of participants. However, as outlined in Table 6 follow up rates varied between centres. The number of infants from Milan with one-year data was especially low because the dates on which most participants' 12-month questionnaires were completed were not available. This made it impossible to determine the age of these infants at the time of data collection. The fact that the baseline characteristics of participants with and without two-year data were similar suggests that loss to follow up is unlikely to have had a significant impact on the results. Nevertheless, parents of children who experience wheezing or other illnesses are less likely to drop out of a study involving contact with paediatric specialists than parents of healthy children. Furthermore, parents with a personal or family history of allergic diseases may be more motivated to participate in a study looking at the prevalence of food allergy.[161] Indeed, at four study centres anonymous data on family history and educational level were collected from 2320 parents who declined participation in the EuroPrevall study. Parents

who agreed to participate had a higher level of education and were more likely to have allergic diseases.[137,140] Given that paternal allergic disease and maternal asthma were associated with wheeze in some models, the prevalence of wheeze in the EuroPrevall cohort may have been higher than in the general population.

Another potential limitation of this analysis is that wheeze prevalence estimates were dependent on parents' understanding of the term wheeze. Studies have shown that this differs widely.[10] Michel et al., for example, used a questionnaire survey to assess parents' understanding of the term wheeze. In a random population sample of 4115 parents, 83.5% correctly defined wheeze as a whistling or squeaking noise. A correct definition was, however, less likely in families of South Asian ethnicity (OR 0.56, 95% CI 0.42-0.75, p<0.001), when parents' first language was not English (OR 0.64, 95% CI 0.48-0.85, p=0.002) and in families from deprived areas (OR 0.60, 95% CI 0.47-0.77). Mothers educated for more than 16 years were more likely to give a correct definition (OR 1.47, 95% CI 1.21-1.79, p<0.001). [162] Maternal education and ethnicity differed between study centres. In some centres more than 10% of mothers had not completed a basic education, compared to less than 1% in others. These differences may have implications for the validity of comparisons between centres. The study questionnaires were, however, translated from English into different languages and verified with back translation to English to minimise the potential for misunderstanding. Furthermore, they were based on the widely used ISAAC questionnaires, which have been validated in many languages for the assessment of wheezing and asthma in school aged children.[22,139]

As outlined in section 2.4, one and two-year data were derived from 12-month, 24-month and symptomatic questionnaires depending on the age at which these questionnaires were completed. Recurrent wheeze (wheeze in both the first and second years of life) was initially proposed as the primary outcome for this analysis. This was to avoid the inclusion of infants with wheeze secondary to a single respiratory tract infection. The prevalence of recurrent wheeze was, however, <1% in three centres (Vilnius, Lodz and Athens), making it difficult to evaluate risk factors for wheeze in these countries. Furthermore, data on wheeze in the first and second years of life was only available in 58% participants. This compares to 73% for data on wheeze in the second year of life. Therefore, wheeze in the second year of life was used as the primary outcome to give a larger sample size and allow adjustment for multiple confounders. Most two-year data was derived from 24-month questionnaires, which referred specifically to wheeze without colds. Therefore, restricting the primary analysis to children with wheeze in the second year of life still avoided inclusion of those with wheeze related only to respiratory tract infections. The study questionnaires could have been improved by asking parents how many episodes of wheeze their child had experienced in the past 12 months. This would have allowed a sensitivity analysis

including only those who had experienced multiple episodes of wheeze to be performed. Finally, the prospective nature of birth cohort studies limits the potential for recall bias. However, given that some questionnaires were completed 6 months after the time period for which they were intended to capture data, recall bias cannot be ruled out.

7.1.4 Future Work

In the EuroPrevall cohort, heterogeneity between centres in terms of both baseline factors and potential risk factors for wheeze was observed. Therefore, the primary multivariable model and sensitivity model one were adjusted for study centre. Study centre was significant suggesting that unmeasured factors were operating in individual centres. Further research is needed to identify these factors, as these may provide opportunities for intervention.

The EuroPrevall birth cohort has recently been followed up at school age as part of the Integrated Approaches to Food Allergy and Allergen Risk Management (iFAAM) study. Follow up in all centres consisted of an online questionnaire to collect data on allergic diseases, skin prick testing, measurement of specific IgE to common food and aeroallergens and collection of DNA. In selected centres, including Southampton, spirometry and measurement of eNO were also undertaken. In children with symptoms suggestive of food allergy and/or evidence of sensitisation to foods, double-blind, placebo-controlled food challenges were performed. iFAAM will therefore allow the relationship between challenge-proven food allergy and asthma at school age to be examined. A link between IgE-positive challenge proven cow's milk allergy in the first year of life and asthma at school age has been demonstrated in a prospective birth cohort study by Saarinen et al. However, the relationship between challenge-proven allergies to other foods and asthma needs to be explored in a prospective manner. Using data collected as part of the EuroPrevall study and iFAAM, it will also be possible to examine the relationship between wheeze in the first two years of life and asthma and food allergy at school age across Europe.

7.1.5 Conclusions

The EuroPrevall birth cohort provides unique data on the prevalence of and risk factors for early childhood wheeze across Europe. Early onset food allergy and infant feeding practices were not associated with wheeze in the second year of life. In keeping with previous studies, lower respiratory tract infections, day care attendance, maternal smoking in infancy and male gender were identified as important risk factors. The relationship between these and wheeze differed between centres, suggesting that additional risk factors may be operating in different countries. Further research is needed to identify these. Meanwhile, preventing respiratory tract infections and minimising postnatal smoke exposure may help to reduce the burden of early childhood wheeze.

7.2 Allergic Disease and Allergic Sensitisation in the UBIOPRED Cohorts

An association between asthma and allergy in childhood has been long recognised.[60,163] Studies have also shown that allergic sensitisation is associated with persistence of asthma into adulthood[164] and is a risk factor for asthma exacerbations in both children and adults.[134] However, no previous studies have looked at how the prevalences of allergic disease and allergic sensitisation differ across the life course in patients with mild to moderate and severe asthma/preschool wheeze.

7.2.1 Allergic Diseases

In the UBIOPRED study, the prevalence of diagnosed eczema was highest in school aged children, followed by preschool children and adults. Of those with eczema, the proportion with active disease did not differ significantly according to age. The majority of preschool and school aged children with eczema developed the condition before the age of 2. However, in most adults the onset of eczema was in later childhood/adulthood. These findings were similar in participants with mild to moderate and severe asthma/wheeze. Previous research has suggested that eczema (atopic dermatitis) is largely a disease of early infancy, which tends to remit in the first few years of life. Illi et al., for example, followed 1314 children in the MAS from birth to 7 years. They found that the cumulative prevalence of atopic dermatitis (AD) by age 2 was 21.5% and that of the children with AD, 43.2% were in remission complete remission by age 3 years, 38.3% had intermittent disease and only 18.7% had persistent AD (characterised by symptoms every year up to 7 years of age).[78] In the UBIOPRED study, however, 64% of school aged children with severe asthma and 70% of school aged children with mild to moderate asthma had active eczema. This suggests that in children with asthma, eczema is not confined to early childhood. Even amongst the adult UBIOPRED cohorts, the prevalence of diagnosed eczema ranged from 29% in the MMAn and SAs/ex cohorts to 35% in the SAn cohort. This is much higher than the prevalence of eczema in population based studies of adults. Recently, for example, Barbarot et al. conducted a large, international, web-based survey to estimate the prevalence of atopic dermatitis in adults.[165] This found that the prevalence of self-reported physician diagnosed AD was 8.4% with a point prevalence of 4.4% (95% CI 4.2-4.6%).

For allergic rhinitis and hay fever, the prevalence of diagnosed disease was lowest in preschool children and highest in school aged children. This was true for participants with mild to moderate and severe disease. Active disease was most common in school aged participants but was reported by over 75% of participants in all cohorts. As expected, onset of disease below the age of 2 years was rare. Although the prevalences of diagnosed allergic rhinitis and hay fever were

lower in adults than school aged children, they were still up to 56% (in non-smoking adults with severe asthma) and 51% (in non-smoking adults with mild to moderate asthma), respectively. As reported in the baseline adult UBIOPRED paper, these prevalences were higher than those in the healthy adult control group.[143] Previous research has shown that the prevalence of allergic rhinitis in patients with asthma is higher than that of the general population, with some studies reporting it to be as high as 80%.[166] Furthermore, rhinitis is known to be a powerful predictor of adult-onset asthma, particularly in atopic individuals. Shaaban et al., for example, found that in a cohort of patients from the ECRHS individuals with allergic rhinitis had a 3.65-fold (95% CI 2.37-5.61) greater risk of developing asthma than controls over an 8.8 year period.[167]

Overall, the above findings from the UBIOPRED cohorts indicate that allergic diseases such as eczema and allergic rhinitis are prevalent across the life course in patients with asthma and not just in paediatric patients. Therefore, assessment and management of allergic co-morbidities is important in all asthma patients.

7.2.2 Food Allergy

For participants with severe asthma, the prevalence of any food allergy was 8.3-10 times higher in adults than school aged children with asthma (depending on the cut-off values used to define food allergy). The prevalence of any food allergy was also significantly higher in school aged children with severe asthma compared to preschool children with severe wheeze. Similar results were seen when comparing the prevalence of food allergies in the mild to moderate asthma/preschool wheeze cohorts. For food allergy as a whole, there were no significant differences in prevalence between children with preschool wheeze and adults with asthma. However, some allergies were more common in preschool children than adults. For example, the prevalence of possible milk allergy was 4.0% in the SW cohort compared to 0.7% in the SAn cohort (0.025) and the prevalence of possible egg allergy was 4.0% in the SW cohort compared to 0.3% in the SAn cohort (0.005). Previous research has demonstrated that cow's milk and hen's egg allergy tend to develop in the first two years of life and resolve by school age.[83,84] Therefore, these findings are not surprising. However, a higher prevalence of milk and egg allergies in school aged children compared to preschool children (in both those with mild to moderate and severe disease) was an unexpected finding. Possible explanations for this are that some school aged children had outgrown their allergies but had persistent sensitisation or that children with asthma are more likely to have co-existing food allergy than children with preschool wheeze. Regarding cow's milk allergy, some cases are non-IgE mediated. However, in the UBIOPRED study, only IgE mediated allergy was considered. This may also explain why age related prevalence patterns of food allergy differed from previous studies. Another possible explanation, which warrants further

investigation, is that food allergies are more likely to persist into later childhood in those with asthma than in the general population. Numerous studies have demonstrated that allergic sensitisation is a risk factor for persistent asthma.[60] However, no studies have investigated whether the reverse is true in relation to food allergy i.e. that asthma is a risk factor for persistent food allergy.

7.2.3 Atopy

For both the mild to moderate and severe asthma/preschool wheeze cohorts, the prevalence of atopy was lower in preschool children than school aged children and adults. For example, the prevalence of atopy in school aged children with severe asthma was over 2 times higher than in preschool children with severe wheeze (42.9 vs 88.8%, p <0.001). In those with mild to moderate disease, the prevalence of atopy was identical in school aged children and adults (89.7%). In school aged children with severe asthma the prevalence of atopy was not statistically different to that in non-smoking adults with severe asthma. The prevalence of atopy was, however, higher in the SA and SAn cohorts compared to the SAs/ex cohort. This may be due to the fact that in smokers atopy plays a less important role in the development and expression of asthma. Although the prevalence of atopy did not differ between school aged children and non-smoking adults with severe asthma, the median number of sensitisations was significantly higher in school aged children (4 vs 1, p<0.001). Previous research has demonstrated that quantification of atopy (either by the level of specific IgE, the size of skin test wheals or the number of positive tests) is important because it more accurately predicts outcomes such as exacerbations than use of arbitrary cut-offs to define atopy.[59] This finding therefore suggests that allergic sensitisation may be of greater clinical significance in school age children than adults. Nevertheless, the fact that the majority of adults in both the mild to moderate and severe non-smoking cohorts (in whom the mean age at asthma diagnosis was 19.9 and 24.0 years, respectively) had atopy, challenges the perception that adult-onset asthma is predominantly non-allergic.[168]

7.2.4 Comparing Participants with Severe and Mild to Moderate Disease

In the UBIOPRED study, there were largely no differences between participants with mild to moderate and severe asthma/preschool wheeze in terms of eczema, allergic rhinitis, hay fever, food allergy and atopy prevalence. A higher prevalence of allergic diseases/atopy was expected in those with severe disease given that allergic sensitisation is implicated in the development of asthma and previous studies have demonstrated an association between atopy and worse asthma outcomes. [168] In the adult UBIOPRED cohorts, the prevalence of atopy and median number of allergic sensitisations was in fact higher in adults with mild to moderate asthma than in non-

smoking adults with severe asthma and smokers/ex-smokers with severe asthma. Differences between the MMA and SAs/ex cohorts may be due to the influence of smoking on asthma severity. However, a higher prevalence of atopy in the MMAn cohort than the SAn cohort and the fact there were no differences between the severe and mild to moderate cohorts in children suggests that atopy is not a major driver of asthma severity.

7.2.5 Strengths and Limitations

A major strength of the UBIOPRED study is that all cohorts were assessed in the same way, allowing direct comparison of outcomes between preschool children, school aged children and adults. In this analysis food allergy as a whole and specific food allergies were evaluated. It was important to consider individual food allergies because the age of onset of food allergy depends on the food.[83] A limitation of this analysis is that double-blind, placebo-controlled food challenges were not used to confirm food allergies. However, use of specific IgE and skin prick test results in conjunction with clinical history is more reliable than use of self-report or physician diagnosis alone. Furthermore, a sensitivity analysis, using higher cut-off values for skin prick testing and IgE results, was performed. Studies have previously demonstrated that using higher cut-off values improves the specificity of skin prick testing and IgE measurement.[86,88] The cut-off values used in this analysis (5mm for skin prick testing and 10KU/l for specific IgE) are lower than those at which 100% specificity has been demonstrated. However, using higher values may have resulted in a higher false negative rate of food allergy.

Regarding the assessment of atopy, use of both skin prick testing (SPT) and specific IgE is a strength of analysis. It has previously been proposed that the two should be used in a complementary manner because SPT is more sensitive and specific IgE is more specific. Frith et al., for example, demonstrated that among 47 children with severe, therapy-resistant asthma there was 20% discordance between SPT and specific IgE results for individual allergens; most commonly with SPT being negative and specific IgE being positive.[169] Studies have also shown that quantification of atopy is important.[59] Therefore, in this analysis the median number of allergens to which participants were sensitised was calculated.

Recall bias is an important limitation of the UBIOPRED study, particularly for the adult cohorts who are likely to have poor recollection of conditions they developed in childhood. Another noteworthy limitation is that participants with mild to moderate wheeze/asthma were recruited from general and respiratory clinics. Therefore, they are unlikely to be representative of patients with mild to moderate wheeze/asthma who are managed in primary care.[142] This limits the external validity of the results and may explain why fewer differences than expected were

observed between the mild to moderate and severe cohorts. In addition to including patients managed in primary care, the UBIOPRED study could have been improved by recruiting healthy controls into both the adult and paediatric cohorts. This would have allowed differences in the prevalences of allergic disease and allergic sensitisation between those with and without asthma to be explored across the life course.

7.2.6 Future Work

In this analysis, allergic sensitisation and atopy were defined according to standard allergy tests. There were few significant differences between those with mild to moderate and severe asthma/preschool wheeze. The relationship between allergen component clusters and asthma/wheeze severity in the UBIOPRED cohorts has been evaluated by Fontanella et al. (manuscript in preparation). Although allergic sensitisation patterns did not differ between patients with mild to moderate and severe disease, patterns of connectivity and interactions between component-specific IgEs were identified as predictors of asthma severity in school aged children and adults with asthma. Replicating these findings in other cohorts would help to determine whether applying network analysis techniques to component-resolved diagnostics data would provide useful prognostic information in patients with asthma.

7.2.7 Conclusions

This analysis has explored the prevalence of allergic diseases and allergic sensitisation across the life course in patients with asthma and preschool wheeze. The prevalence of allergic diseases and allergic sensitisation was also compared between participants with mild to moderate and severe asthma/preschool. Although the prevalence of allergic diseases and atopy were highest in school aged children, allergic rhinitis, hay fever and atopy were also highly prevalent in adults with asthma. Furthermore, the prevalences of allergic disease and atopy did not differ according to asthma/wheeze severity in children or adults. Ongoing allergen exposure in sensitised individuals is associated with an increased risk of exacerbations and may cause steroid resistance. Allergy testing is therefore recommended in severe asthma.[169] However, it is not routinely performed in all patients with asthma. The BTS/SIGN guideline on the management of asthma, for example, states that specific IgE and allergen skin prick tests may be of value in selected patients with asthma.[115] The findings of this analysis suggest, however, that allergy testing may be beneficial in all asthma patients in order to identify potential triggers for symptoms and exacerbations.

7.3 Exacerbations in the UBIOPRED Cohorts

Asthma exacerbations are a major cause of morbidity in both children and adults with asthma[128] and are associated with a considerable social and economic burden.[12] Therefore, identifying risk factors for exacerbations is a key research priority. It is increasingly recognised that asthma is a heterogeneous condition consisting of multiple different phenotypes.[100] This analysis aimed to determine whether rates of future exacerbations differ between clinical clusters of patients with severe asthma/preschool wheeze or between patients with different patterns of allergic sensitisation. Risk factors for future severe exacerbations in the severe UBIOPRED cohorts were also explored.

7.3.1 Exacerbation Rates

It was hypothesised that rates of future exacerbations would differ between clinical clusters and ISAC component atopy clusters of patients in the severe UBIOPRED cohorts. For paediatric participants, higher rates of moderate exacerbations were observed in clinical cluster 2 and clinical cluster 6 compared to some of the other clusters. The median rate of moderate exacerbations was, however, 0 for all clinical clusters. Therefore, these differences are unlikely to be of clinical significance. For paediatric participants, no other differences in exacerbation rates were observed between clinical clusters or ISAC component atopy clusters.

For adults, exacerbation rates did not differ between clinical clusters when taking all types of exacerbations into account. However, clinical cluster 1 had a lower rate of non-hospitalised severe exacerbations compared to the other 3 clinical clusters (the median exacerbation rate was 0 for cluster 1, compared to 0.8 for cluster 2, 1 for cluster 3 and 0.8 for cluster 4, p=0.036). A lower exacerbation rate might be expected in clinical cluster 1 given that this consisted of patients with well-controlled asthma, normal lung function and minimal corticosteroid use. Differences in the rates of severe hospitalised exacerbations between clinical clusters were also statistically significant. However, once again, the median rate exacerbation rate was 0 for all clusters so these differences may not be clinically significant.

For some types of exacerbations, exacerbation rates differed according to patterns of allergic sensitisation in adults. Previous studies have demonstrated that allergic sensitisation is associated with an increased risk of asthma exacerbations in adults.[170] It is therefore surprising that the median rate of all exacerbations was lower in adults with miscellaneous sensitisation compared to non-sensitised adults (1 vs 1.7 exacerbations per year, p=0.002). The median rate of non-hospitalised severe exacerbations was also higher in non-sensitised adults compared to those with grass pollen sensitisation (1 vs 0 exacerbations per year, p=0.026) and miscellaneous

sensitisation (1 vs 0 exacerbations per year, p=0.026). A possible explanation for these findings is that sensitised individuals had low levels of allergen exposure or had asymptomatic sensitisation. Indeed, a significant number of individuals with positive allergy tests have no evidence of allergic disease.[134] The results may also be confounded by medication use. For example, adults with allergic sensitisation may have been taking higher doses of corticosteroids at baseline putting them at lower risk of exacerbations.

7.3.2 Risk Factors for Exacerbations

In agreement with previous studies, a history of asthma exacerbations [111,116-118] and poor asthma control[119,120,122] were identified as risk factors for future asthma exacerbations in all of the severe UBIOPRED cohorts. For every additional exacerbation in the previous year, the risk of future severe exacerbations increased by a factor of 1.13 (95% CI 1.03-1.24, p=0.007) in children and 1.18 (95% CI 1.07-1.31, p=0.002) in adults. Research in adults has shown that a history of severe exacerbations confers a higher risk of future exacerbations than a history of moderate exacerbations.[118] Therefore, these risk estimates may have been higher if only severe exacerbations (rather than any exacerbations) in the previous year had been taken into account. Regarding asthma control, in the severe paediatric cohorts, a higher asthma control z-score (indicative of better asthma control) was associated with a lower risk of future severe exacerbations (IRR 0.57, 95% CI 0.43-0.76, p<0.001) whilst in the severe adult cohorts a higher asthma control z-score (indicative of worse asthma control) was associated with higher risk of future severe exacerbations (IRR 1.37, 95% CI 1.16-1.61, p<0.001). These findings suggest that optimising asthma treatment to achieve adequate disease control is essential in order to reduce the risk of future exacerbations. Physicians should also ensure that they explore patients' recent exacerbation history at every clinical encounter and intervene when necessary to minimise the risk of repeat episodes.

Other risk factors for wheeze/asthma exacerbations in the UBIOPRED cohorts varied according to age. In preschool children with severe wheeze, stress as a reported symptom trigger was a risk factor for future severe exacerbations. This finding is plausible given that in asthma, emotional stress may accentuate inflammatory responses to allergic and infectious triggers through neuroimmunological mechanisms.[171] Furthermore, stress in preschool children may reflect stress in the home environment, which may adversely affect parents' coping mechanisms and ability to manage their children's asthma.[171]

Previous research has shown that boys are more likely to suffer from acute asthma before puberty but thereafter the gender difference reverses.[128] When school aged children with severe

asthma from the UBIOPRED study were analysed separately, girls were 3.17 times more likely than boys to experience future severe exacerbations (95% CI 1.78-5.64, p <0.001). In combined analysis of preschool and school aged children, being female was also a risk factor for future severe exacerbations (IRR 1.79, 95% CI 1.11-2.91, p=0.018). This may be due to the fact that the mean age of participants in the severe asthma cohort was 12.2 years and the severe asthma cohort was larger than the preschool wheeze cohort. In preschool children with severe wheeze, there was a trend towards girls being at lower risk of future severe exacerbations than boys in univariate analysis. However, this finding was not statistically significant (IRR 0.63, 95% CI 0.28-1.43, p=0.270).

Surprisingly, a higher quality of life was associated with an increased risk of future severe exacerbations in school aged children with severe asthma (IRR 1.56, 95% CI 1.07-2.29, p=0.022). A possible explanation for this is that patients who do not perceive their asthma to be problematic discontinue regular treatment putting themselves at increased risk of asthma exacerbations. Alternatively, some patients with asthma believe that periodic exacerbations are to be expected[130] and thus may report good quality of life despite frequent exacerbations.

None of the paediatric clinical clusters were associated with future severe exacerbations in univariate or multivariable analysis. This was unexpected given that one of the variables used to generate the clusters was the exacerbation rate at baseline. Previous research has, however, suggested that clusters of asthma patients do not relate to clinical outcomes such as exacerbations rates and treatment requirements.[104,105] Regarding atopic sensitisation, children in ISAC component atopy cluster 4 (with miscellaneous sensitisation) were 2 times more likely to experience future severe exacerbations than non-sensitised children (IRR 2.01, 95% CI 1.06-3.81, p=0.034). Allergic sensitisation and atopy were not associated with future exacerbations when defined according to standard tests (skin prick testing and specific IgE to whole allergen extracts). This finding suggests that performing component resolved diagnostics in children with asthma may be beneficial as it may help to identify those at increased risk of exacerbations. It has previously been proposed that quantification of atopy more accurately predicts asthma exacerbations than information on the presence or absence of atopy alone and that atopy encompasses multiple phenotypes which differ in their association with asthma outcomes.[134] For example, when Lazic et al. used a machine learning approach to cluster children from the MAAS and Isle of Wight birth cohorts into different classes of atopic sensitisation, they found that children in the class with sensitivity to a wide variety of allergens had poorer lung function, higher eNO levels and most hospital admissions for asthma (aOR 15.3, 95% CI 5.3-44.1 in MAAS and aOR 2.5, 95% CI 1.3-4.7 in IoW).[71]

In the adult UBIOPRED cohorts, a previous ICU admission (IRR 1.37, 95% CI 1.01-1.86, p=0.045) and having symptoms triggered by pets (IRR 1.43, 95% CI 1.08-1.98, p=0.013) were identified as risk factors for future severe asthma exacerbations. Given the latter association, it is surprising that patients who were sensitised and exposed to cat or dog were not at increased risk of exacerbations compared to those who were not sensitised/exposed. Indeed, previous studies have demonstrated that a combination of allergen sensitisation and high allergen exposure is associated with increased asthma severity in both children and adults.[134] Murray et al., for example, demonstrated that among 60 adults hospitalised with asthma, being exposed and sensitised to house dust mite, cat or dog was an independent risk factor for hospital admission (OR 2.3, 95% CI 1.0-5.4, p <0.001).[170] The risk of hospital admission was further increased in adults with allergic sensitisation, high allergen exposure and evidence of viral infection (OR 8.4, 95% CI 2.1-32.8, p=0.002).[170] It is not surprising that a previous ICU admission is a risk factor for future severe asthma exacerbations as patients who have required ICU admission are more likely to have poorly controlled asthma.

Whilst children in ISAC component atopy cluster 4 (with miscellaneous sensitisation) were at increased risk of future severe exacerbations, adults in this ISAC component atopy cluster were at a lower risk of future severe exacerbations (IRR 0.57, 95% CI 0.39-0.84, p=0.005). A possible explanation for this discrepancy is that the miscellaneous sensitisation groups comprised individuals with positive responses to a few of a broad range of components. Therefore, specific sensitisation patterns may have differed between children and adults in this ISAC component atopy cluster. Children and adults may also have had different levels of allergen exposure, which would influence the risk of future exacerbations. Similar to in children, none of the adult clinical clusters were associated with an increased risk of future severe exacerbations.

7.3.3 Strengths and Limitations

In the UBIOPRED study preschool children, school aged children and adults were analysed in a similar way allowing direct comparison of outcomes between age groups. Inclusion of preschool children is a particular strength of the UBIOPRED study because few previous studies utilising asthma clusters have included preschool children and there is limited evidence regarding risk factors for exacerbations in children with preschool wheeze.[115] Furthermore, most studies of preschool wheeze have utilised birth cohorts, whereas in the UBIOPRED study children with preschool wheeze were recruited on the basis of a consensus definition.[142] The fact that all children in the severe wheeze and severe asthma cohorts had to have been under the care of a respiratory paediatrician for at least 6 months prior to study enrolment is both a strength and limitation of the UBIOPRED study. The advantage of this is that diagnoses of severe asthma and

severe preschool wheeze are likely to have been accurate. However, it also means that exacerbation rates may not have been representative of real life. For all of the severe cohorts, the number of exacerbations in the previous year at baseline was higher than the number of exacerbations per year during follow up. For example, for school aged children with severe asthma the median number of exacerbations in the previous year at baseline was 4 with an interquartile range of 2-5. This compares to a median exacerbation rate of 1.3 (interquartile range 0-3.9) during follow up. It has previously been recognised that rates of exacerbations in clinical trials are likely to be lower than in real life because patients enrolled in trials are usually more compliant with treatment and are assessed at regular intervals to assess asthma control.[130]

The results of this analysis may also have been subject to recall bias. Although participants were asked to contact their local study centre if they experienced an exacerbation, most data on exacerbations were collected at longitudinal follow up visits. Participants' recollection of what treatment they received for exacerbations which occurred 12-18 months previously may be unreliable. Indeed, in some cases, exacerbations could not be classified due to missing data. For all patients, specific doses of controller medications, including inhaled and oral corticosteroids, were not available on tranSMART. Therefore, it was not possible to adjust for medication use in multivariable analysis.

Another potential limitation of the UBIOPRED study is that only participants with severe disease were followed up. Although asthma exacerbations are more common in severe asthma,[107] they occur across all levels of disease severity.[113] Therefore, studies evaluating risk factors for asthma exacerbations may be more informative if they include patients with both mild to moderate severe disease. Follow up of only participants with severe disease in the UBIOPRED study also means that results relating to the clinical and ISAC component atopy clusters need to be interpreted with caution because the clusters were generated from participants with both mild to moderate and severe disease. This may in fact explain why overall rates of exacerbations did not differ between clusters. Nevertheless, the percentage of participants in each cluster at baseline was similar to the percentage of participants in each cluster at follow up, suggesting that patients with severe disease were represented equally in all clusters. It is also important to note that some of the clinical clusters, particularly the paediatric clusters, consisted of small numbers of participants. For example, paediatric clinical cluster 6 comprised only 13 participants, of whom only 8 had severe asthma/preschool wheeze. Similarly, paediatric ISAC component atopy cluster 3 comprised only 17 participants of whom only 8 had severe disease. Therefore, the conclusions drawn by this work on how future exacerbation rates differ between clinical clusters and ISAC component atopy clusters of patients with asthma/preschool wheeze need to be validated in a larger study.

7.3.4 Future Work

Previous research has that suggested that clinical phenotypes lack temporal stability and may be of limited clinical benefit in patients with asthma.[172] Furthermore, exacerbation prediction tools based on clinical variables have previously been developed but have had limited success.[111] It has therefore been proposed that asthma endotypes (which arise through common pathophysiological mechanisms) need to be identified.[100] In this analysis rates of future exacerbations did not differ significantly between clinical and allergic sensitisation clusters of patients with asthma/wheeze. In the adult UBIOPRED cohorts, it has already been demonstrated that sputum proteomics and transcriptomics differ between clusters based on clinical parameters.[151] Further work is needed to determine whether novel biomarkers such as proteomics and transcriptomics can identify patients at increased risk of asthma exacerbations. This may ultimately enable the development of powerful prediction tools combining clinical variables and biomarkers.

7.3.5 Conclusions

Identifying patients at increased risk of asthma exacerbations is important because it may lead to improved disease management and reduced morbidity. In the UBIOPRED cohorts, a higher number of exacerbations in the previous year and poor asthma control were important risk factors for future severe exacerbations across the life course. Female gender was also a risk factor for exacerbations in children, whilst having a previous ICU admission and having symptoms triggered by pets were risk factors in adults. This thesis has expanded on previous research by demonstrating that, overall, rates of future severe exacerbations did not differ between clinical clusters or allergic sensitisation clusters of patients asthma/preschool wheeze. Further research is needed to determine whether novel biomarkers can predict asthma exacerbations. In the meantime, it is essential that patients with a history of exacerbations are engaged with clinical follow up and that asthma control is optimised in all patients.

Appendix A

A.1 EuroPrevall Ethics Approval

NHS

North & Mid Hampshire Local Research Ethics Committee
1ST Floor, Regents Park Surgery
Park Street, Shirley
Southampton
SO16 4RJ

CPW/sks

23 September 2005

Doctor Graham Roberts
Clinical Senior Lecturer Paediatric Allergy & Respiratory Medicine
University of Southampton
Child Health, Level F South block (803)
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SO16 6YD

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Application Submission: submissions@gp-j82203.nhs.uk

Dear Doctor Roberts

Full title of study: **The Prevalence of food allergy and weaning practises in a birth cohort of UK infants.**
REC reference number: **05/Q1703/34**

Thank you for your letter of 18 August 2005, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information was considered at the meeting of the Sub-Committee of the REC held on 23 September 2005. A list of the members who were present at the meeting is attached.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised.

Conditions of approval

The favourable opinion is given provided that you comply with the conditions set out in the attached document. You are advised to study the conditions carefully.

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Application	4.1	14 June 2005
Investigator CV Chief investigator CV		01 April 2005
Investigator CV Principal Investigator CV		
Protocol		14 June 2005
Covering Letter		13 June 2005
Questionnaire 30 month questionnaire		
Questionnaire 24 month questionnaire		
Questionnaire 12 month questionnaire		
Questionnaire Physician questionnaire for symptomatic visit		
Questionnaire Visit questionnaire (symptomatic and control infant/child)		

An advisory committee to Hampshire and Isle of Wight Strategic Health Authority

Appendix A

Questionnaire At birth		
Questionnaire Intake, at birth	2 (amended)	08 August 2005
Questionnaire 12 month	2 (amended)	08 August 2005
Questionnaire 24 month	2 (amended)	08 August 2005
Questionnaire 30 month	2 (amended)	08 August 2005
Questionnaire Visit	2 (amended)	08 August 2005
Sample Diary/Patient Card	1	14 June 2005
Advertisement Poster/flyer	1	14 June 2005
Letter of invitation to participant	1	24 May 2005
GP/Consultant Information Sheets	1	24 May 2005
GP/Consultant Information Sheets	2 (amended)	05 August 2005
Participant Information Sheet	2 (amended)	14 August 2005
Participant Information Sheet	1	24 May 2005
Participant Consent Form	1	14 June 2005
Participant Consent Form	2 (amended)	14 August 2005
Response to Request for Further Information		18 August 2005
Evaluation summary report for integrated project		
PIFA study protocol	2 (amended)	14 August 2005
Physical examination	2 (amended)	08 August 2005
Food Diary Record	2 (amended)	
Food Allergy poster	Marked version	
Study Introduction Sheet	2 (amended)	
Letter from fund provider		06 May 2005

Research governance approval

The study should not commence at any NHS site until the local Principal Investigator has obtained final research governance approval from the R&D Department for the relevant NHS care organisation.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

05/Q1703/34	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Jane Ogden-Swift
Chair

Email: GM.E.hio-au.SWHRECA@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments.*

Standard approval conditions,
Site approval form

Copy to: Southampton University Hospitals Trust
R + D Office
Trust Management Offices, Mailpoint 18
Tremona road, Southampton
SO16 6YD

Appendix A

North & Mid Hampshire Local Research Ethics Committee

Attendance at Sub-Committee of the REC meeting on 23 September 2005

Committee Members:

Name	*Profession*	*Present?*	*Notes*
Mr Paul Gartell	LREC Member	Yes	
Mrs Sue Wolstenholme	LREC member	Yes	
Dr Paul O Halloran	LREC Member	Yes	

North & Mid Hampshire Local Research Ethics Committee

LIST OF SITES WITH A FAVOURABLE ETHICAL OPINION

For all studies requiring site-specific assessment, this form is issued by the main REC to the Chief Investigator and sponsor with the favourable opinion letter and following subsequent notifications from site assessors. For issue 2 onwards, all sites with a favourable opinion are listed, adding the new sites approved.

REC reference number:	05/Q1703/34	Issue number:	1	Date of issue:	23 September 2005
Chief Investigator:	Doctor Graham Roberts				
Full title of study:	The Prevalence of food allergy and weaning practises in a birth cohort of UK infants.				

This study was given a favourable ethical opinion by North & Mid Hampshire Local Research Ethics Committee on 23 September 2005. The favourable opinion is extended to each of the sites listed below. The research may commence at each NHS site when management approval from the relevant NHS care organisation has been confirmed.

Principal Investigator	*Post*	*Research site*	*Site assessor*	*Date of favourable opinion for this site*	*Notes [1]*
Dr Keith Foote		Family Services Division Paediatrics RHCH Romsey Rd Winchester HANTS	North & Mid Hampshire Local Research Ethics Committee	23/09/2005	

Approved by the Chair on behalf of the REC:

..... (Signature of Chair/Administrator)

(delete as applicable)

..... (Name)

A.2 EuroPrevall Wheeze Manuscript Analysis Plan

Introduction and Background

Wheeze is a major health problem in the first two years of life with many children who wheeze, later developing atopic asthma. Given that food allergy is one of the first manifestations of atopy, infants with food allergy may be more likely to develop early onset, persistent wheeze. Numerous factors are, however, implicated in the aetiology of preschool wheeze, including early life nutrition and exposure to cigarette smoke.

We propose to assess the risk factors for wheeze in the first two years of life within the pan-European EuroPrevall birth cohort, focusing on food allergy, breastfeeding, complementary feeding, and peri-natal smoke exposure. This will be undertaken using Poisson regression, comparing infants with persistent wheeze (at 12 and 24 months) to a never wheezed group. Infants with wheeze at only one time point will not be included in order to reduce the chance of including those with wheeze secondary to a single viral respiratory tract infection. Wheeze at only 12 months and wheeze at only 24 months will, however, be included in a secondary analysis.

A number of specific hypotheses will be tested. The primary hypothesis will be that early onset food allergy (defined by a positive double-blind, placebo-controlled food challenge (DBPCFC)) is an important risk factor for early onset, persistent wheeze. Additionally, we will look at the effects of the duration of breastfeeding, the timing of onset of complementary feeding, the overlap between breastfeeding and complementary feeding, maternal smoking during pregnancy and infancy and paternal smoking. Planned additional exposure variables will include antenatal factors and maternal and paternal factors such as atopy.

Data from UK EuroPrevall cohort

Risk factors for wheeze in the first two years of life have already been analysed using data from the UK EuroPrevall cohort focusing on antenatal maternal nutrition and smoking, postnatal infant smoke exposure and breastfeeding. This was undertaken using univariate logistic regression on a number of chosen variables from the data set against wheeze at 12 months, wheeze at 24 months, wheeze at 12 months only, wheeze at 24 months only and persistent wheezing at 12 and 24 months. Maternal smoking rates were low in the UK cohort. However, antenatal passive smoking was found to be a significant risk factor for persistent wheeze (OR 1.95, 95% CI 1.05-3.62, p=0.042). Longer duration of breastfeeding was protective against wheeze in the first year of life (OR 0.94 per week, 95% CI 0.97-1.00, p=0.005).

We propose to repeat and extend this analysis in the complete European EuroPrevall cohort. This will allow us to focus on food allergy as a potential risk factor for wheeze and provide the power to look for potential protective effects of breastfeeding and increased overlap between breastfeeding and complementary feeding. It will also allow us to determine whether antenatal or postnatal smoke exposure is most deleterious.

Hypotheses

Primary hypothesis: Food allergy presenting in the first two years of life is an independent risk factor for persistent wheeze in early childhood.

Secondary hypotheses:

- Longer breastfeeding is associated with less persistent wheeze.
- Increased overlap between breastfeeding and complementary feeding is associated with less persistent wheeze.
- Earlier onset of complementary feeding is associated with less persistent wheeze.
- Maternal smoking during pregnancy and infancy plus other household smokers are all risk factors for persistent wheeze.
- Birth length, weight and gestation influence the expression of wheeze in the first two years of life.

Dataset

The EuroPrevall birth cohort dataset is held at the Charité University Medical Centre, Berlin.

The analysis will include all participants:

i. satisfying the inclusion criteria
ii. not satisfying the exclusion criteria
iii. who were at least 24 months at the cut off point for data entry
iv. whose data was entered into the study database by the cut-off point

Statistical Analysis

Analysis will be undertaken using SPSS v22 and STATA SE v13.

Data Checking

Variables in the dataset will be checked to determine whether they lie within acceptable limits and whether they are in the correct format.

Appendix A

Analysis Plan

1. Generate dataset for this analysis.
2. Validate dataset by assessing ranges, means, medians and proportions of each variable, as appropriate.
3. Undertake data cleaning as required.
4. Generate variables for age at onset of complementary feeding and overlap with breastfeeding.
5. Generate variable coding for the outcome variable: never wheezed, wheeze at 12 months, wheeze at 24 months and persistent wheeze (wheeze at 12 and 24 months).
6. Assess the association between exposures (e.g. challenge diagnosed food allergy, nutrition, smoking) and persistent wheeze using Poisson regression to model relative risk. A 5% level of statistical significance will be used.
7. Exposures related to the outcome with a p-value of 0.1 or less will be entered into a backward deletion multivariable analysis model.

Authorship of Manuscript

Writing group: Anna Selby*, Alasdair Munro, Graham Roberts**,

Other authors: Maximum 2 from each centre plus coordination centre.

*First author and **last/corresponding author.

References

McBride D, Keil T, Grabenhenrich L, Dubakiene R, Drasutiene G, Fiocchi A, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. Pediatr Allergy Immunol 2012; 23 (3): 230-9.

Keil T, McBride D, Grimshaw K, Niggemann B, Xepapadaki P, Zannikos K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. Allergy 2010; 65 (4): 482-490.

A.3 EuroPrevall 12-Month Follow-up Questionnaire

EuroPrevall

12-Month Follow-up Questionnaire

Please fill in EuroPrevall Participant-ID first

1	8	0				

EuroPrevall–ID

A. Feeding your child

1.	Do you or did you ever breastfeed your child?	○ Yes, but not breastfeeding anymore	○ Yes, still breastfeeding (go to Q5)	○ No (go to Q5)				
2.-4.	How old was your child when you stopped breastfeeding him/her?	☐☐ Months	or ☐☐ Weeks	or ☐☐ Days				
5.	If you delivered in a hospital, was there anything that interfered with your ability to breastfeed your baby, because the baby was in special care, given light treatment or for other reasons?	○ Yes	○ No					
6.	How do you feed your child at present?	○ Breast-fed only	○ Both breast and formula fed	○ Breast-fed plus solid foods	○ Both breast and formula fed plus solid foods	○ Bottle fed infant formula only	○ Bottle fed infant formula plus solid foods	○ Not breast or formula fed anymore
7.	How many times on average do you feed your child (breast/bottle/solid foods) over a 24-hour period? (do not count snacks)	☐☐ times						
8.	Has your child **ever** received any infant formula?	○ Yes	○ No (Go to Q85)					

	Which of the following types of infant formula has your child received?												
		For **each** type of formula please list the **approximate number of days per month** the child received that formula (if daily = 30)											
	Age when received (in months)	If the annual telephone interview has already been completed please use the appropriate child's age (months)											
		1 (13) (25) mo	**2 (14) (26) mo**	**3 (15) (27) mo**	**4 (16) (28) mo**	**5 (17) (29) mo**	**6 (18) (30) mo**	**7 (19) mo**	**8 (20) mo**	**9 (21) mo**	**10 (22) mo**	**11 (23) mo**	**12 (24) mo**
9.	Normal cow's milk formula												
21.	Normal soy milk formula												
33.	Hypo-allergenic (modified) formula												
45.	Other (please specify) 45a_____												
57.	Other (please specify) 57a_____												

69.	What type of infant formula are you giving your child at the moment?	_____ (Formula/bottle milk name)
	Did the following influence you in your choice of formula?	
70.	Because of a family history of allergies	○ Yes ○ No
71.	Child prefers the taste of this formula	○ Yes ○ No
72.	Prescribed/recommended by my doctor or other health professional	○ Yes ○ No
73.	Price of formula	○ Yes ○ No
74.	This was the brand used in hospital	○ Yes ○ No

75.	Did you ever add anything to the formula in the bottle?	○ Yes ○ No (Go to Q80)
	If yes, what did you add?	
76.	Sugar	○ Yes ○ No
77.	Honey	○ Yes ○ No
78.	Infant cereal	○ Yes ○ No
79.	Something else (please specify)	_____

80.	How do you clean your child's bottles and teats?	
80.	Wash them, then boil them in water	○ Yes ○ No
81.	Wash them, then rinse them in hot water	○ Yes ○ No
81a.	Wash them in a dishwashing machine	○ Yes ○ No
82.	Wash them, then soak them in sterilizing solution in a unit/tank	○ Yes ○ No
83.	Wash them, then put them in a steam sterilizing unit	○ Yes ○ No
84.	Does the bottle nipple come in contact with an adult's mouth before being given to the child?	○ Yes, regularly ○ Yes, occasionally ○ No ○ Don't know
	What is the pacifier/dummy made of?	
84a.	Latex (yellow)	○ Yes ○ No
84b.	Silicon (transparent)	○ Yes ○ No

Questions about your breastfeeding. If you <u>never</u> breastfed you child, please go to <u>Q154</u>		
85.	When breastfeeding, have you used, or do you use, any creams or oils on your breasts and/or nipples?	○ Yes ○ No (Go to Q90)
	Do they contain any of the following oils?	
86.	Soy oil	○ Yes ○ No ○ Don't know
87.	Peanut oil	○ Yes ○ No ○ Don't know
88.	Soy lethicin oil	○ Yes ○ No ○ Don't know

	If any other oil, please specify the brand and/or name of the cream or oil	
89.	Brand/Name of cream/oil	_____

	Before breastfeeding do you or did you wash your breasts/nipples with any of the following?		
90.	A little expressed breast milk	○ Yes	○ No
91.	Soap and water	○ Yes	○ No
91a.	Just water	○ Yes	○ No
92.	Antiseptic wipes/solution	○ Yes	○ No
93.	Nothing	○ Yes	○ No

When breastfeeding, do you or did you eat, and/or increase, avoid or limit your intake of certain foods or drinks for any reason?
From the list below, please indicate your eating habits whilst breastfeeding

		Do/did you eat this food?		In comparison to your eating habits before your breastfeeding, do or did you…? …eat the same amount?	…eat an increased amount?	…limit your intake?	…avoid it altogether?
94.	Milk and other dairy products	○ Yes	○ No	○	○	○	○
96.	Soy products (e.g. milk, tofu, sprouts)	○ Yes	○ No	○	○	○	○
98.	Eggs	○ Yes	○ No	○	○	○	○
100.	Peanuts	○ Yes	○ No	○	○	○	○
102.	Tree nuts	○ Yes	○ No	○	○	○	○
104.	Seeds (e.g. sesame, sunflower, poppy)	○ Yes	○ No	○	○	○	○
106.	Fish	○ Yes	○ No	○	○	○	○
108.	Shellfish	○ Yes	○ No	○	○	○	○
110.	Cereals and cereal products	○ Yes	○ No	○	○	○	○
112.	Vegetables	○ Yes	○ No	○	○	○	○
114.	Legumes	○ Yes	○ No	○	○	○	○
116.	Fruit	○ Yes	○ No	○	○	○	○

		Do/did you eat this food?	In comparison to your eating habits before your breastfeeding, do or did you…?			
			…eat the same amount?	…eat an increased amount?	…limit your intake?	…avoid it altogether?
118.	Meat and meat products (including poultry)	○ Yes ○ No	○	○	○	○
120.	Coffee and tea	○ Yes ○ No	○	○	○	○
122.	Alcohol	○ Yes ○ No	○	○	○	○
124.	Confectionaries (e.g. chocolate, candies)	○ Yes ○ No	○	○	○	○
126.	Fish liver oil	○ Yes ○ No	○	○	○	○
128.	Probiotics (specify brand) 128a_____	○ Yes ○ No	○	○	○	○
130.	Other (please specify) 130a_____		○	○	○	○
132.	Other (please specify) 132a_____		○	○	○	○

Supplements

When **breastfeeding**, do you or did you take any of the following **supplements**?

		Yes, regularly at least several times a week	Yes, for a specific episode	Yes, occasionally	No
134.	Folic acid	○	○	○	○
135.	Multivitamins	○	○	○	○
136.	Vitamin D (specifically)	○	○	○	○
137.	If yes, what was the average dose of Vitamin D?	☐☐☐☐☐ I.U.			
138.	Fish oil capsules	○	○	○	○
139.	Other supplements (please specify) 139a_____	○	○	○	
140.	Other supplements (please specify) 140a_____	○	○	○	

Medications

When **breastfeeding**, do you or did you take any of the following **medications**?

		Yes, regularly (at least several times a week)	Yes, for a specific episode	Yes, occasionally	No
141.	Antibiotics	○	○	○	○
142.	Aspirin / Paracetamol	○	○	○	○

143.	Anti-inflammatory, e.g. ibuprofen, nurofen	○	○	○	○
144.	Medication for reflux diseases	○	○	○	○
145.	Insulin	○	○	○	○
146.	Oral antidiabetics	○	○	○	○
147.	Corticosteriods	○	○	○	○
147a.	local	○	○	○	○
147b.	oral	○	○	○	○
147c.	inhaled	○	○	○	○
148.	Medication for high blood pressure	○	○	○	○
149.	Medications for other conditions (please specify medication or if unknown, the condition) 149a_____	○	○	○	
150.	Medications for other conditions (please specify medication or if unknown, the condition) 150a_____	○	○	○	

Alternative medicines (e.g. homeopathic, plant, Chinese, etc. Please specify)					
When **breastfeeding**, do you or did you take any of the following **alternative medicines**?					
		Regularly at least several times a week	For a specific episode	Occasionally	No
151.	(Name of medicine) 151a_____	○	○	○	○
152.	(Name of medicine) 152a_____	○	○	○	○
153.	(Name of medicine) 153a_____	○	○	○	○

B. Your child's eating habits

154.	How many meals of solid food a day does your child eat (do not count snacks)?	☐☐ times		
155.	How would you describe the variety of foods that your child generally eats? Does s/he:	○ eat most types of food	○ eat a reasonable variety of foods	○ The child is a fussy eater

	Do the following factors influence you when deciding what types of solid foods to give your child? **(Please answer for ALL Factors)**			
156.	Nutritional content	○ Yes	○ No	○ Don't know
157.	Taste/child's preferences	○ Yes	○ No	○ Don't know

158.	Recommended by doctors or other health professional	○ Yes	○ No	○ Don't know
159.	Organic ingredients	○ Yes	○ No	○ Don't know
160.	Price	○ Yes	○ No	○ Don't know
161.	Want to reduce risk of allergy	○ Yes	○ No	○ Don't know
162.	Other (please specify)			

The following is a list of foods. Please describe for each food listed:

- whether your child has tried this food in the last year
- how old he/she was when he/she first tried this food
- whether he/she has eaten this food in the last 3 days
- whether you avoid giving the child this food and the reason(s) why

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
163a.	Cow's milk*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q181a)	○ Yes ○ No	○ Yes ○ No
163.	pasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
169.	unpasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
175.	ultra heat treated (UHT)	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
181a.	Cheese*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q199)	○ Yes ○ No	○ Yes ○ No
181.	unpasteurised soft cheese	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
187.	pasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
193.	processed	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
199.	Yoghurt or fromage frais*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
205.	Yoghurt with bifidus, bioactive or probiotic drinks	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
211.	Butter	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
217.	Goat's milk/cheese*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
223.	Sheep's milk/cheese*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
229a.	Soy*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q247 a)	○ Yes ○ No	○ Yes ○ No
229.	Soy milk	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
235.	Tofu	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
241.	Soy sprouts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
247a.	Eggs*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q265 a)	○ Yes ○ No	○ Yes ○ No
247.	boiled	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
253.	scrambled	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
259.	in baked products	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
265a.	Peanuts*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q289 a)	○ Yes ○ No	○ Yes ○ No
265.	roasted peanuts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
271.	peanut butter	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
277.	Peanuts in the shell	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
283.	Peanuts as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
289a.	Tree nuts*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q379a)	○ Yes ○ No	○ Yes ○ No
289.	Hazelnuts Whole nut	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
295.	Hazelnuts as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
301.	Hazelnuts as nut spread	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
307.	Hazelnuts as praline	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
313.	Walnuts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
319.	Macadamia	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
325.	Almonds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
331.	Almonds as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
337.	Almonds as marzipan	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
343.	Pistachios	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
349.	Pecans	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
355.	Brazil nut	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
361.	Brazil nut as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
367.	Cashews	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
373.	Cashews as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
379a.	Seeds*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q403a)	○ Yes ○ No	○ Yes ○ No
379.	Mustard seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
385.	Sunflower seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
391.	Sesame seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
397.	Poppy seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
403a.	Fish and seafood*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q421a)	○ Yes ○ No	○ Yes ○ No
403.	White fish, e.g. cod	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
409.	Oily fish, e.g. salmon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
415.	Crustaceans, e.g. shrimp	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
421a.	Cereal/cereal products*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q469a)	○ Yes ○ No	○ Yes ○ No
421.	Wheat	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
427.	Rye	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
433.	Barley	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
439.	Oats	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
445.	Buckwheat	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
451.	Bread	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
457.	Noodles	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
463.	Rice	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
469a.	Vegetables*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q553a)	○ Yes ○ No	○ Yes ○ No
469.	Corn	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
475.	Carrots	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
481.	Celery	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
487.	Potatoes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
493.	Avocado	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
499.	Tomatoes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
505.	Squash	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
511.	Beets	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
517.	Onions	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
523.	Garlic	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
529.	Beans	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
535.	Peas	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
541.	Chickpeas	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
547.	Lentils	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
553a.	Fruits*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q617a)	○ Yes ○ No	○ Yes ○ No
553.	Peaches	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
559.	Apricots	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
565.	Kiwi	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
571.	Apples	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
577.	Banana	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
583.	Oranges	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
599.	Pears	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
605.	Grapes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given in last 3 days	Avoid giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
611.	Melon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
617a.	Meats*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No (Go to Q659)	○ Yes ○ No	○ Yes ○ No
617.	Beef	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
623.	Lamb	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
629.	Pork	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
635.	Sausages	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
641.	Bacon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
647.	Ham	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
653.	Poultry	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
659.	How many tins of peanuts or how much peanut butter is consumed in the household, even if the child does not eat it?	☐☐.☐ Medium-sized (250 gm) jars or 250 gm tins/month					

660.	Does your child receive vitamins or other supplements?	○ Yes ○ No (Go to Q684)				
	Which vitamins or supplements does he/she receive?					
661.	**Multivitamins**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
663.	Were they recommended by a health professional?	○ Yes ○ No				
664.	**Vitamin D**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week

666.	Name of Vitamin-D product	_____				
667.	Average dose of Vitamin-D	☐☐☐☐☐ I.U.				
668.	Was this recommended by a health professional?	○ Yes	○ No			
669.	**Fluoride**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
671.	Was this recommended by a health professional?	○ Yes	○ No			
672.	**Fish oil**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
674.	Was this recommended by a health professional?	○ Yes	○ No			

675.	**Fish oil capsules**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
677.	Were they recommended by a health professional?	○ Yes	○ No			
678.	**Others** (please list) 678a_____	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
680.	Was this recommended by a health professional?	○ Yes	○ No			
681.	**Others** (please list) 681a_____	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
683.	Was this recommended by a health professional?	○ Yes	○ No			

684.	Does your child use a pacifier/dummy?	○ Yes	○ No (Go to Q688)
	When yes, what is the pacifier/dummy made of?		
685.	Latex (yellow)	○ Yes	○ No
686.	Silicon (transparent)	○ Yes	○ No

687.	Has the pacifier/dummy ever come in contact with an adult's mouth before being given to the child?	○ Yes, regularly	○ Yes, occasionally	○ No	○ Don't know

C. Your child's health

688.	Has your child ever had a rash which was coming and going for at least 6 months? **(Do not count a regular nappy rash)**	○ Yes	○ No
689.	Has your child had a rash or eczema that has lasted for at least 7 days or more? **(Do not count a regular nappy rash)**	○ Yes	○ No
690.	Have any of these rashes at any time affected the fold of the elbows, behind the knees, in front of the ankles, on the cheeks or around the neck, ears or eyes? **(Do not count a regular nappy rash)**	○ Yes	○ No (Go to Q693)
691.	At what age (months) did this rash begin?	☐☐ month/s	
692.	Was this rash itchy?	○ Yes	○ No

693.	Has your child had any of the following skin problems?		
693.	Dry or red patches on the skin	○ Yes	○ No (Go to Q695)
694.	At what age (months) did this begin?	☐☐ month/s	
695.	Swollen lips	○ Yes	○ No (Go to Q697)
696.	At what age (months) did this begin?	☐☐ month/s	
697.	Urticaria/hives	○ Yes	○ No (Go to Q699)
698.	At what age (months) did this begin?	☐☐ month/s	

699.	Has your child had an episode of vomiting without any fever?			
699.	Only spit up	○ No	○ Yes, occasionally	○ Yes, often
700.	Repeated vomiting	○ No	○ Yes, occasionally	○ Yes, often
701.	Has your child had colic? (sudden continuous crying, bloated stomach, steadily passing gas, cramping and pulling up legs)	○ No (go to Q703)	○ Yes	○ Yes, occasionally

702.	At what age did it start?	☐☐ month/s
703.	On average, how many days per week does your child have a bowel movement? (every day=7)	☐ days/week (if <7, go to Q705)
704.	(only if Q703 is answered with 7) If your child has a bowel movement every day, how many times per day on average?	☐☐ times/day
705.	Has your child had diarrhoea (at least 3 very loose stools per day) **without any fever**?	○ No (Go to Q707) ○ Yes, only once ○ Yes, occasionally ○ Yes, often
706.	If yes, did it contain blood?	○ No ○ Yes, only once ○ Yes, occasionally ○ Yes, often
707.	Has your child been constipated? (i.e. could not have a bowel movement more than once without aid, such as manipulation, medications, etc.)	○ No ○ Yes, only once ○ Yes, occasionally ○ Yes, often

708.	**In the last *12* months**, has the child had a problem with sneezing or a runny, or blocked nose when s/he did not have a cold or the flu?	○ Yes ○ No (Go to Q722)
709.	Has this nose problem been accompanied by itchy-watery eyes?	○ Yes ○ No
710. – 721.	In which of the **past *12* months** did this nose problem occur? (Please tick any which apply)	Jan ☐ Feb ☐ Mar ☐ Apr ☐ May ☐ Jun ☐ Jul ☐ Aug ☐ Sep ☐ Oct ☐ Nov ☐ Dec ☐
722.	**In the last *12* months**, has a doctor ever diagnosed the child as having hay fever?	○ Yes ○ No
723.	**In the last *12* months**, has a doctor ever diagnosed the child as having an allergy to house dust?	○ Yes ○ No
724.	**In the last *12* months**, has your child had an allergic reaction when in contact with animals? *(sneezing episodes with multiple sneezes, runny, blocked nose without cold or fever, with watery, red or swollen eyes.)*	○ Yes ○ No (Go to Q732)

725.	**In the last *12* months**, has a doctor ever diagnosed the child as having an allergy to animals?	○ Yes	○ No	
	To which animal(s) has an allergy been diagnosed??			
726.	Dogs	○ Yes	○ No	
727.	Cats	○ Yes	○ No	
728.	Birds	○ Yes	○ No	
729.	Rodents	○ Yes	○ No	
730.	Horses	○ Yes	○ No	
731.	Others (please specify)	_____		
732.	Has the child had an allergic reaction to latex in the last 12 months *(urticaria, especially around mouth, e.g. after use of dummy or blowing up a ballon)*	○ Yes	○ No (Go to Q734)	
733.	Has a doctor diagnosed the child as having an allergy to latex in the last 12 months?	○ Yes	○ No	
734.	Has your child had an adverse reaction to bee or wasp stings in the last 12 months? *(local itching, urticaria rash or swelling, breathing difficulties or collapse within 2 hours of being stung by bee or wasp)*	○ Yes, just local *(local itching, local urticaria rash or local swelling)*	○ Yes, local and symptomatic *(generalized urticaria, angio-oedema, breathing difficulties or collapse within 2 hours of being stung by bee or wasp)*	○ No (Go to 736)
735.	Has a doctor diagnosed the child as having an allergy to bees or wasps in the last 12 months?	○ Yes	○ No	

736.	**In the last *12* months,** has your child had wheezing or whistling in the chest?	○ Yes	○ No
737.	**In the last *12* months,** has your child's chest sounded wheezy during or after exercise?	○ Yes	○ No
738.	**In the last *12* months,** has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?	○ Yes	○ No
739.	**In the last *12* months,** did a doctor ever diagnose asthma in your child?	○ Yes	○ No
	In the last *12* months, have any of the following medications been used?		

740.	bronchodilators	○ Yes	○ No
741.	antihistamines	○ Yes	○ No
742.	Corticosteroids		
	oral	○ Yes	○ No
	inhaled	○ Yes	○ No
743.	Does your child have adverse reactions to any foods, such as eczema, breathing problems or gastrointestinal problems?	○ Yes (please complete **Food Allergy Form**)	○ No (Go to Q744)

Food Allergy Form
is a separate Excel form with pull-down menus for each food group and then for each group of symptoms

744.	Has the child had allergy testing in the last 12 months? *(IgE, SPT, food challenge, etc.)*	○ Yes	○ No (Go to Q749)	○ Don't know (Go to Q749)
	Which tests were positive?			
745.	Serum IgE (blood test)	○ Positive	○ Negative	○ Don't know
746.	Skin Prick Test	○ Positive	○ Negative	○ Don't know
747.	Food challenge	○ Positive	○ Negative	○ Don't know
748.	Other (please specify) 748a_____	○ Positive	○ Negative	○ Don't know

749.	Has your child had injection immunotherapy (desensitisation) in the last 12 months?	○ Yes	○ No (Go to Q750)	○ Don't know (Go to Q750)
	For which allergens?	749a.		
		749b.		
		749c.		
750.	Has your child had oral tolerance induction therapy in the last 12 months?	○ Yes	○ No (Go to Q751)	○ Don't know (Go to Q751)

	For which allergens?	750a.
		750b.
		750c.

751.	How often has your child had any of the following infections **in the last 12 months**?	
751.	Upper respiratory infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
752.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q753) ○ Don't know (Go to Q753)
	If yes, please list by which antibiotic(s)	752a.
		752b.
		752c.
		752d.
		752e.
753.	Lower respiratory infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
754.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q755) ○ Don't know (Go to Q755)
	If yes, please list by which antibiotic(s)	754a.
		754b.
		754c.
		754d.
		754e.
755.	Wheeze in association with an upper respiratory infection (cold)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
756.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q757) ○ Don't know (Go to Q757)
	If yes, please list by which antibiotic(s)	756a.
		756b.
		756c.
		756d.
		756e.
757.	Bronchiolitis (bronchitis)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)

758.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q759) ○ Don't know (Go to Q759)
	If yes, please list by which antibiotic(s)	758a. 758b. 758c. 758d. 758e.
759.	Middle ear infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
760.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q761) ○ Don't know (Go to Q761)
	If yes, please list by which antibiotic(s)	760a. 760b. 760c. 760d. 760e.
761.	Gastrointestinal illness (diarrhoea/vomiting)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
762.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q763) ○ Don't know (Go to Q763)
	If yes, please list by which antibiotic(s)	762a. 762b. 762c. 762d. 762e.
763.	Other infections (list) 763a.----------	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
764.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q765) ○ Don't know (Go to Q765)
	If yes, please list by which antibiotic(s)	764a. 764b. 764c.

		764d.
		764e.
765.	Other infections (list) 765a.----------	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
766.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q767) ○ Don't know (Go to Q767)
	If yes, please list by which antibiotic(s)	766a.
		766b.
		766c.
		766d.
		766e.
767.	Approximately how many times has your child received antibiotics **in the last 12 months?**	☐☐ times (if "0" go to Q771)
768.	How old was your child when s/he received the first antibiotic?	☐☐ months
769.	When did s/he receive the last antibiotic?	☐☐ a. Month ☐☐☐☐ b. Year
770.	What was the name of this antibiotic?	_____
771.	Have you taken your child to a doctor because of any of the following illnesses?	
771.	Weight loss	○ Yes ○ No
772.	Failure to thrive	○ Yes ○ No
773.	Other (not including well-baby checks or immunizations, please specify)	_____
774-775.	What is your child's weight and height?	☐☐.☐ kg Weight ☐☐☐ cm Height At age ☐☐ months
	Has your child received any of the following medications?	
776.	Aspirin	○ Yes ○ No
777.	Paracetemol	○ Yes ○ No
778.	Anti-inflammatories (e.g. Ibuprofen, Nurofen)	○ Yes ○ No
779.	Corticosteroids	
779a.	local	○ Yes ○ No

779b.	oral	○ Yes ○ No
779c.	inhaled	○ Yes ○ No
780.	Reflux medications	○ Yes ○ No
781.	Cough syrup and/or expectorants	○ Yes ○ No
782.	Other (please specify)	_____
783.	Other (please specify)	_____

784.	Has your child been given any homeopathic or natural supplements, including Chinese medications?	○ Yes ○ No (Go to Q801) ○ Don't know (Go to Q801)
	If yes, give the name of the supplement, the age when it was started, how long it was given and whether the child is still receiving it.	
785.	**Name** of supplement (please specify)	_____
786.	Age when started	☐☐ months
787.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
788.	Still given?	○ Yes ○ No
789.	**Name** of supplement (please specify)	_____
790.	Age when it started	☐☐ months
791.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
792.	Still given?	○ Yes ○ No
793.	**Name** of supplement (please specify)	_____
794.	Age when it started	☐☐ months
795.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
796.	Still given?	○ Yes ○ No
797.	**Name** of supplement (please specify)	_____

798.	Age when it started	☐☐ months
799.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
800.	Still given?	○ Yes ○ No

801.	Has your child received any vaccinations?	○ Yes ○ No (go to Q946)							
	What vaccinations has your child received and date(s) received? (from vaccination record)								
802-809.	6-Pack (polio, diphtheria, whooping cough, tetanus, Hepatitis B, H. influenza B)	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
810-817.	5-Pack (polio, diphtheria, whooping cough, tetanus, H. influenza B)	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
818-825.	Polio	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
826-833.	Diphtheria	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
834-841.	Whooping cough	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
842-849.	Tetanus	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
850-857.	Hepatitis B	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
858-865.	Hemophilus influenza B	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
866-873.	MMR (measles, mumps, rubella)	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
874-881.	Measles	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
882-889.	Mumps	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year
890-897.	Rubella (German measles)	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year	☐☐ month ☐☐ year

898-905.	Meningitis C	month year	month year	month year	month year
906-913.	Tuberculosis (BCG)	month year	month year	month year	month year
914-921.	Pneumococcal (Pneumovax)	month year	month year	month year	month year
922-929.	Chicken pox	month year	month year	month year	month year
930-937.	Other (please specify) 937a_____	month year	month year	month year	month year
938-945.	Other (please specify) 938a_____	month year	month year	month year	month year

946.	What do you use to bathe your child?	○ warm water only	○ baby soap or liquid/wash	○ regular soap or liquid/wash	○ other
947.	Other (please specify)	_____			
948.	Do you use any creams, lotions or powders on your child's skin?	○ Yes	○ No (Go to Q 951)		
949.	If yes, please specify brand / product name	_____			
950.	Type	☐ a. Cream/lotion	☐ b. Powder		

D. You and your household

951.	In the past 12 months, has have there been any new allergies or allergic symptoms in the child's mother, father or blood-related sibling(s)?	○ Yes (please complete the appropriate **"Changes in Allergy"** Form)		○ No (please ensure all **Baseline Allergy Questionnaires** are completed)
952.	Do you (mother) smoke?	○ Yes, daily	○ Yes, occasionally	○ No
953.	Does anyone else smoke inside your home?	○ Yes, daily	○ Yes, occasionally	○ No
954.	Is your child exposed to tobacco smoke outside the home? (for example at grandparents or other relatives, baby sitter)	○ Yes, daily	○ Yes, occasionally	○ No

955.	Do you work in paid employment at the moment?	○ Yes ○ Yes, but currently on maternity leave (Go to Q957) ○ No (Go to Q957)
956.	How old was your child when you first went back to work?	☐☐ months
957.	Does your child attend day care or a nursery?	○ Yes ○ No (Go to Q988)
958.	How many hours a week on average does your child attend day care or nursery?	☐☐ **hours**
959.	How old was your child when he/she first started day care?	☐☐ months
960.	What type of day care does your child attend?	○ Childminder ○ Nursery/crèche
961.	Approximately how many other children are cared for by the childminder or attend the nursery/crèche?	☐☐ children
962.	Does the childminder OR nursery/crèche have a pet(s)	○ Yes ○ No (Go to Q988)

963-987. If yes, please choose up to 5 animals from the list, list the number of each and where they are allowed (multiple answers possible)

	Number of each	Where are they allowed? Where child sleeps	Living room	Kitchen	Only outside the house
Dogs		☐	☐	☐	☐
Cats		☐	☐	☐	☐
Birds		☐	☐	☐	☐
Rodents		☐	☐	☐	☐
Horses		☐	☐	☐	☐
Goats		☐	☐	☐	☐
Cows		☐	☐	☐	☐
Chickens		☐	☐	☐	☐
Pigs		☐	☐	☐	☐
Reptiles		☐	☐	☐	☐
Insects		☐	☐	☐	☐
Fish		☐	☐	☐	☐

988.	Have you moved in the past 12 months?	○ Yes ○ No (Go to Q996)
989.	What is your new post code?	☐☐☐☐☐☐☐☐
990.	If you don't know your new post code, in what is the name of the suburb or town in which do you now live?	_____
991.	In what type of area do you now live?	○ Urban ○ Rural
992.	What is the approximate population of your city or town?	☐☐ ☐☐☐ ☐☐☐
993.	If you now live in a rural area, do you live on a farm?	○ Yes ○ No
994.	Do you now live on or near a main road where heavy vehicles (trucks, buses) pass by?	○ Yes ○ No
995.	Are there any areas of mould in your flat or house?	○ Yes ○ No ○ Don't know

996.	What main type of flooring is in the room where your child sleeps?	○ Carpet ○ Wooden, laminate, parquet ○ Linoleum or vinyl tiles ○ Ceramic / terracotta tiles or stone ○ Sea-grass or coir-type matting ○ Other
997.	If other, please specify	_____

998.	What kind of mattress does your child sleep on?	○ Raw hair ○ Foam ○ Synthetic (other than foam) ○ Feather ○ Other
999.	If other, please specify	_____
1000.	Does your baby's mattress have a plastic surface or cover?	○ Yes ○ No
1001.	What type of bedding is on your child's bed?	○ blankets ○ Feather or down-filled quilt ○ Synthetic-filled quilt ○ Other (please specify)
1002.	If other, please specify	_____

	If your infant has had more than one type of permanent bed in the last 12 months (i.e. a cot or cradle and then a child's crib), please give the information for the previous bed	
998a.	What kind of mattress did your child sleep on?	○ Raw hair ○ Foam ○ Synthetic (other than foam) ○ Feather ○ Other
999a.	If other, please specify	_____
1000a.	Did your baby's mattress have a plastic surface or cover?	○ Yes ○ No
1001a.	What type of bedding was on your child's bed?	○ blankets ○ Feather or down-filled quilt ○ Synthetic-filled quilt ○ Other (please specify)
1002a.	If other, please specify	_____

1003.	Does your baby regularly (at least once a week) share a bed with you?	○ Yes ○ No
1004-1012.	How do you wash your child's clothes and bedding (please check on all that apply)	☐ Reg. Washing soap ☐ Baby (mild) washing soap ☐ Rinse with softener ☐ Rinse with vinegar ☐ Extra rinse ☐ Air-dry clothes ☐ Dry in the dryer ☐ Iron clothes or bedding ☐ Other (please specify)
1013.	If other, please specify	_____

1014.	Do you have any animals?	○ Yes ○ No (Go to Q1040)
1015-1039.	If yes, please choose up to 5 animals from the list, list the number of each and where they are allowed (multiple answers possible)	

	Number of each	Where are they allowed? Where child sleeps	Living room	Kitchen	Only outside the house
Dogs		☐	☐	☐	☐
Cats		☐	☐	☐	☐
Birds		☐	☐	☐	☐
Rodents		☐	☐	☐	☐
Horses		☐	☐	☐	☐
Goats		☐	☐	☐	☐
Cows		☐	☐	☐	☐
Chickens		☐	☐	☐	☐
Pigs		☐	☐	☐	☐
Reptiles		☐	☐	☐	☐
Insects		☐	☐	☐	☐
Fish		☐	☐	☐	☐

1040.	What do you usually use to clean your kitchen work surfaces?	○ Non-bactericidal cleaning product ○ Bactericidal cleaning product ○ None of these ○ Don't know
1041.	What do you usually use to clean the table where you eat?	○ Antibacterial spray cleaner ○ Soap and water ○ Just water ○ None of these
1042.	How often do you wash your hands after eating peanuts or peanut butter before handling this child?	○ Always ○ Most of the time ○ Sometimes ○ Seldom / never ○ Do not eat peanuts / peanut butter
1043.	How many adults live in the household?	☐☐ (Number)
1044.	How many children live in the household? (including this child)	☐☐ (Number)
1045.	How many bedrooms does your home have, including the child's room and guest room?	○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8+

Mother's Quality of life

We would like for you to answer a few questions about your current health state. Please indicate which statements best describe your own health state today.

QoL1. Mobility

- I have no problems in walking about ❑
- I have some problems in walking about ❑
- I am confined to bed ❑

QoL2. Self-Care

- I have no problems with self-care ❑
- I have some problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

QoL3. Usual Activities (*e.g. work, study, housework, family or leisure activities)*

- I have no problems with performing my usual activities ❑
- I have some problems with performing my usual activities ❑
- I am unable to perform my usual activities ❑

QoL4. Pain/Discomfort

- I have no pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have extreme pain or discomfort ❑

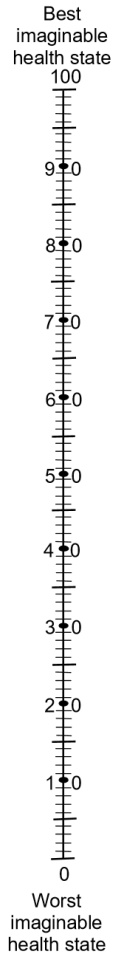
QoL5. Anxiety/Depression

- I am not anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am extremely anxious or depressed ❑

QoL6. To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion.

Your own health state today



	Date Questionnaire completed	☐☐ Day ☐☐ Month ☐☐☐☐ Year

If any symptoms of an **allergy** were related to intake of particular foods, **please remember** to complete the

Food Allergy Form

If there have been **any changes in allergies** in the mother, father or blood-related sibling, **please remember** to complete the

Change in Allergies Questionnaire

for that person/those persons

Thank mother / father for their participation!

A.4 EuroPrevall 24-Month Follow-up Questionnaire and Symptomatic Questionnaire age 13-24 Months

EuroPrevall

24-Month Follow-up Questionnaire Symptomatic Questionnaire age 13 – 24 months

Please fill in EuroPrevall Participant-ID first

		0					

EuroPrevall–ID

Is this a:		
O 24-month telephone interview	**O Symptomatic questionnaire**	
	O symptomatic child	**O control child**

All questions relate to when the child was 13 – 24 months old

A. Feeding your child

1.	Do you or did you ever breastfeed your child since she/he was 12 months old?	O Yes, but not breastfeeding anymore	O Yes, still breastfeeding (go to Q5)	O No (go to Q5)				
2.-4.	How old was your child when you stopped breastfeeding him/her?	☐☐ Months	or ☐☐ Weeks	or ☐☐ Days				
5.	If you delivered in a hospital, was there anything that interfered with your ability to breastfeed your baby, because the baby was in special care, given light treatment or for other reasons?	O Yes	O No					
6.	How do you feed your child at present?	O Breast-fed only	O Both breast and formula fed	O Breast-fed plus solid foods	O Both breast and formula fed plus solid foods	O Bottle fed infant formula only	O Bottle fed infant formula plus solid foods	O Not breast or formula fed anymore
7.	How many times on average do you feed your child (breast/bottle/solid foods) over a 24-hour period? (do not count snacks)	☐☐ times						
8.	Has your child **ever** received any infant formula since she/he was 12 months old?	O Yes	O No (Go to Q80)					

	Which of the following types of infant formula has your child received?												
		For **each** type of formula please list the **approximate number of days per month** the child received that formula (if daily = 30)											
	Age when received (in months)	**13 mo**	**14 mo**	**15 mo**	**16 mo**	**17 mo**	**18 mo**	**19 mo**	**20 mo**	**21 mo**	**22 mo**	**23 mo**	**24 mo**
9.	Normal cow's milk formula												
21.	Normal soy milk formula												
33.	Hypo-allergenic (modified) formula												
45.	Other (please specify) 45a_____												
57.	Other (please specify) 57a_____												

69.	What type of infant formula are you giving your child at the moment?	_____ (Formula/bottle milk name)
	Did the following influence you in your choice of formula?	
70.	Because of a family history of allergies	○ Yes ○ No
71.	Child prefers the taste of this formula	○ Yes ○ No
72.	Prescribed/recommended by my doctor or other health professional	○ Yes ○ No
73.	Price of formula	○ Yes ○ No
74.	This was the brand used in hospital	○ Yes ○ No

75.	Did you ever add anything to the formula in the bottle?	○ Yes ○ No (Go to Q80)
	If yes, what did you add?	
76.	Sugar	○ Yes ○ No
77.	Honey	○ Yes ○ No

78.	Infant cereal	○ Yes ○ No
79.	Something else (please specify)	_____

80.	If your child drinks or drank from a bottle, how do/did you clean your child's bottles and teats? (answer all that apply)	
80.	Wash them, then boil them in water	○ Yes ○ No
81.	Wash them, then rinse them in hot water	○ Yes ○ No
81a.	Wash them in a dishwashing machine	○ Yes ○ No
82.	Wash them, then soak them in sterilizing solution in a unit/tank	○ Yes ○ No
83.	Wash them, then put them in a steam sterilizing unit	○ Yes ○ No
84.	Does the bottle nipple come in contact with an adult's mouth before being given to the child?	○ Yes, regularly ○ Yes, occasionally ○ No ○ Don't know
	What is the bottle nipple made of?	
84a.	Latex (yellow)	○ Yes ○ No
84b.	Silicon (transparent)	○ Yes ○ No

	Questions about your breastfeeding. If you <u>never</u> breastfed your child, or stopped breastfeeding before the child was 12 months old, please go to <u>Q154</u>	
85.	When breastfeeding, have you used, or do you use, any creams or oils on your breasts and/or nipples?	○ Yes ○ No (Go to Q90)
	Do they contain any of the following oils?	
86.	Soy oil	○ Yes ○ No ○ Don't know
87.	Peanut oil	○ Yes ○ No ○ Don't know
88.	Soy lecithin oil	○ Yes ○ No ○ Don't know
	If any other oil, please specify the brand and/or name of the cream or oil	
89.	Brand/Name of cream/oil	_____

	Before breastfeeding do you or did you wash your breasts/nipples with any of the following?	
90.	A little expressed breast milk	○ Yes ○ No

91.	Soap and water	○ Yes	○ No
91a.	Just water	○ Yes	○ No
92.	Antiseptic wipes/solution	○ Yes	○ No
93.	Nothing	○ Yes	○ No

When breastfeeding, do you or did you eat, and/or increase, avoid or limit your intake of certain foods or drinks for any reason?
From the list below, please indicate your eating habits whilst breastfeeding

		Do/did you eat this food?		In comparison to your eating habits before your breastfeeding, do or did you…?			
				…eat the same amount?	…eat an increased amount?	…limit your intake?	…avoid it altogether?
94.	Milk and other dairy products	○ Yes	○ No	○	○	○	○
96.	Soy products (e.g. milk, tofu, sprouts)	○ Yes	○ No	○	○	○	○
98.	Eggs	○ Yes	○ No	○	○	○	○
100.	Peanuts	○ Yes	○ No	○	○	○	○
102.	Tree nuts	○ Yes	○ No	○	○	○	○
104.	Seeds (e.g. sesame, sunflower, poppy)	○ Yes	○ No	○	○	○	○
106.	Fish	○ Yes	○ No	○	○	○	○
108.	Shellfish	○ Yes	○ No	○	○	○	○
110.	Cereals and cereal products	○ Yes	○ No	○	○	○	○
112.	Vegetables	○ Yes	○ No	○	○	○	○
114.	Legumes	○ Yes	○ No	○	○	○	○
116.	Fruit	○ Yes	○ No	○	○	○	○
118.	Meat and meat products (including poultry)	○ Yes	○ No	○	○	○	○
120.	Coffee and tea	○ Yes	○ No	○	○	○	○
122.	Alcohol	○ Yes	○ No	○	○	○	○
124.	Confectionaries (e.g. chocolate, candies)	○ Yes	○ No	○	○	○	○
126.	Fish liver oil	○ Yes	○ No	○	○	○	○

		Do/did you eat this food?	In comparison to your eating habits before your breastfeeding, do or did you…?			
			…eat the same amount?	…eat an increased amount?	…limit your intake?	…avoid it altogether?
128.	Probiotics (specify brand) 128a_____	○ Yes ○ No	○	○	○	○
130.	Other (please specify) 130a_____		○	○	○	○
132.	Other (please specify) 132a_____		○	○	○	○

Supplements

When **breastfeeding**, do you or did you take any of the following **supplements**?

		Yes, regularly at least several times a week	Yes, for a specific episode	Yes, occasionally	No
134.	Folic acid	○	○	○	○
135.	Multivitamins	○	○	○	○
136.	Vitamin D (specifically)	○	○	○	○
137.	If yes, what was the average dose of Vitamin D?	☐☐☐☐☐ I.U.			
138.	Fish oil capsules	○	○	○	○
139.	Other supplements (please specify) 139a_____	○	○	○	
140.	Other supplements (please specify) 140a_____	○	○	○	

Medications

When **breastfeeding**, do you or did you take any of the following **medications**?

		Yes, regularly (at least several times a week)	Yes, for a specific episode	Yes, occasionally	No
141.	Antibiotics	○	○	○	○
142.	Aspirin / Paracetamol	○	○	○	○
143.	Anti-inflammatory, e.g. ibuprofen, nurofen	○	○	○	○
144.	Medication for reflux diseases	○	○	○	○
145.	Insulin	○	○	○	○
146.	Oral antidiabetics	○	○	○	○
147.	Corticosteriods	○	○	○	○
147a.	topical	○	○	○	○
147b.	oral	○	○	○	○
147c.	inhaled	○	○	○	○

148.	Medication for high blood pressure	○	○	○	○
149.	Medications for other conditions (please specify medication or if unknown, the condition) 149a_____	○	○	○	
150.	Medications for other conditions (please specify medication or if unknown, the condition) 150a_____	○	○	○	

Alternative medicines (e.g. homeopathic, plant, Chinese, etc. Please specify)					
When **breastfeeding**, do you or did you take any of the following **alternative medicines**?					
		Regularly at least several times a week	For a specific episode	Occasionally	No
151.	(Name of medicine) 151a_____	○	○	○	○
152.	(Name of medicine) 152a_____	○	○	○	○
153.	(Name of medicine) 153a_____	○	○	○	○

B. Your child's eating habits

154.	How many meals of solid food a day does your child eat (do not count snacks)?	☐☐ times		
155.	How would you describe the variety of foods that your child generally eats? Does s/he:	○ eat most types of food	○ eat a reasonable variety of foods	○ The child is a fussy eater

	Do the following factors influence you when deciding what types of solid foods to give your child? **(Please answer for ALL Factors)**			
156.	Nutritional content	○ Yes	○ No	○ Don't know
157.	Taste/child's preferences	○ Yes	○ No	○ Don't know

158.	Recommended by doctors or other health professional	○ Yes	○ No	○ Don't know
159.	Organic ingredients	○ Yes	○ No	○ Don't know
160.	Price	○ Yes	○ No	○ Don't know
161.	Want to reduce risk of allergy	○ Yes	○ No	○ Don't know
162.	Other (please specify)	_____		

The following is a list of foods. Please describe for each food listed:

- whether your child has tried this food in the last year
- how old he/she was when he/she first tried this food
- whether he/she eats this food regularly (>1 time a week)
- whether you avoid giving the child this food and the reason(s) why

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
163a.	Cow's milk*	○ Yes (Go to Q163) ○ No			○ Yes ○ No (Go to Q181a)	○ Yes ○ No	○ Yes ○ No (Go to Q181a)
163.	pasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
169.	unpasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
175.	ultra heat treated (UHT)	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
181a.	Cheese*	○ Yes (Go to Q181) ○ No			○ Yes ○ No (Go to Q199)	○ Yes ○ No	○ Yes ○ No (Go to Q199)
181.	unpasteurised soft cheese	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
187.	pasteurised	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
193.	processed	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
199.	Yoghurt or fromage frais*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
205.	Yoghurt with bifidus, bioactive or probiotic drinks	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
211.	Butter	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
217.	Goat's milk/cheese*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
223.	Sheep's milk/cheese*	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
229a.	Soy*	○ Yes (Go to Q229) ○ No			○ Yes ○ No (Go to Q247a)	○ Yes ○ No	○ Yes ○ No (Go to Q247a)
229.	Soy milk	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
235.	Tofu	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
241.	Soy sprouts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
247a.	Eggs*	○ Yes (Go to Q247) ○ No			○ Yes ○ No (Go to Q265a)	○ Yes ○ No	○ Yes ○ No (Go to Q265a)
247.	boiled	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
253.	scrambled	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
259.	in baked products	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
265a.	Peanuts*	○ Yes (Go to Q265) ○ No			○ Yes ○ No (Go to Q289a)	○ Yes ○ No	○ Yes ○ No (Go to Q289a)
265.	roasted peanuts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
271.	peanut butter	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
277.	Peanuts in the shell	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
283.	Peanuts as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
289a.	Tree nuts*	○ Yes (Go to Q289) ○ No			○ Yes ○ No (Go to Q379a)	○ Yes ○ No	○ Yes ○ No (Go to Q379a)

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
289.	Hazelnuts Whole nut	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
295.	Hazelnuts as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
301.	Hazelnuts as nut spread	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
307.	Hazelnuts as praline	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
313.	Walnuts	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
319.	Macadamia	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
325.	Almonds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
331.	Almonds as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
337.	Almonds as marzipan	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
343.	Pistachios	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
349.	Pecans	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
355.	Brazil nut	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
361.	Brazil nut as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
367.	Cashews	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
373.	Cashews as ingredient	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
379a.	Seeds*	○ Yes ○ No			○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food (Go to Q379)	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food (Go to Q403a)	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it? (Go to Q403a)
379.	Mustard seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
385.	Sunflower seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
391.	Sesame seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
397.	Poppy seeds	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
403a.	Fish and seafood*	○ Yes (Go to Q403) ○ No			○ Yes ○ No (Go to Q421a)	○ Yes ○ No	○ Yes ○ No (Go to Q421a)
403.	White fish, e.g. cod	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
409.	Oily fish, e.g. salmon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
415.	Crustaceans, e.g. shrimp	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
421a.	Cereal/cereal products*	○ Yes (Go to Q421) ○ No			○ Yes ○ No (Go to Q469a)	○ Yes ○ No	○ Yes ○ No (Go to Q469a)
421.	Wheat	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
427.	Rye	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
433.	Barley	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
439.	Oats	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
445.	Buckwheat	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
451.	Bread	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
457.	Noodles/Pasta	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
463.	Rice	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
469a.	Vegetables*	○ Yes (Go to Q469) ○ No			○ Yes ○ No (Go to Q553a)	○ Yes ○ No	○ Yes ○ No (Go to Q553a)
469.	Corn	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
475.	Carrots	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
481.	Celery	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
487.	Potatoes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
493.	Avocado	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
499.	Tomatoes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
505.	Squash	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
511.	Beets	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
517.	Onions	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
523.	Garlic	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
529.	Beans	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
535.	Peas	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
541.	Chickpeas	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
547.	Lentils	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
553a.	Fruits*	○ Yes (Go to Q553) ○ No			○ Yes ○ No (Go to Q617a)	○ Yes ○ No	○ Yes ○ No (Go to Q617a)
553.	Peaches	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
559.	Apricots	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
565.	Kiwi	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
571.	Apples	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
577.	Banana	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
583.	Oranges	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
599.	Pears	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
605.	Grapes	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
611.	Melon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
617a.	Meats*	○ Yes (Go to Q617) ○ No			○ Yes ○ No (Go to Q659)	○ Yes ○ No	○ Yes ○ No (Go to Q659)
617.	Beef	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

	Type of food All main categories mandatory (*)	Child has tried this food	Age when 1st tried this food	Given regularly (>1 x/week)	Avoid or delayed giving child this food	When avoided, because you think it could cause allergies	When avoided, because the child doesn't tolerate it?
623.	Lamb	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
629.	Pork	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
635.	Processed meats	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
641.	Bacon	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
647.	Ham	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No
653.	Poultry	○ Yes ○ No	☐☐ Month/s	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No	○ Yes ○ No

659.	How often is a medium-sized (250 gm) tin/jar of peanuts / peanut butter is consumed in the household, even if the child does not eat it?	○ 1/week	○ 1 every 2 weeks	○ 1/month	○ 1 every 3 months	○ rarely	○ never	
How often does your child drink one glass of...								
659a.	Plain water (still or sparkling)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659b.	Hot chocolate (chocolate milk) sweetened	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659c.	Fruit juice (undiluted)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659d.	Fruit juice (diluted)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659e.	Sugary drinks (i.e non-diet cola, Fanta, squash etc)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659f.	Low cal / sugar free drinks (including low-cal hot chocolate)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659g.	Sweetened tea	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never

659h.	Unsweetened tea	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659i.	Cow's milk	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659j.	Other (please specify) _____	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
How often does your child eat one child's portion of…								
659l.	Candies (Sweets)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659m	Chocolate	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659n.	Cake or biscuits	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659o.	Crisps (potato chips)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659p.	Ready meals or fast food (i.e. fries, burgers, chicken nuggets, pizza, etc)	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659q	Fruits	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659r.	Vegetables	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never
659s.	Other (please specify) _____	○ >1/day	○ 1/day	○ >1/week	○ 1/week	○ 3 times or less/month	○ rarely	○ never

All questions relate to when the child was 13 – 24 months old

660.	Does your child receive vitamins or other supplements?	○ Yes	○ No (Go to Q684)			
	Which vitamins or supplements does he/she receive?					
661.	**Multivitamins**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
663.	Were they recommended by a health professional?	○ Yes	○ No			
664.						

	Vitamin D	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
665a.		If not receiving anymore, age when stopped ☐☐ Month/s				
666.	Name of Vitamin-D product	_____				
667.	Average dose of Vitamin-D	☐☐☐☐☐ I.U.				
668.	Was this recommended by a health professional?	○ Yes	○ No			
669.	**Fluoride**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
671.	Was this recommended by a health professional?	○ Yes	○ No			
672.	**Fish oil**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
674.	Was this recommended by a health professional?	○ Yes	○ No			

675.	**Fish oil capsules**	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
677.	Were they recommended by a health professional?	○ Yes	○ No			
678.	**Others** (please list) 678a_____	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
680.	Was this recommended by a health professional?	○ Yes	○ No			
681.	**Others** (please list) 681a_____	Age when first given ☐☐ Month/s	○ Daily	○ Often during the week	○ 1-2 times a week	○ Less than once a week
683.	Was this recommended by a health professional?	○ Yes	○ No			

684.	Does your child use a pacifier/dummy?	○ Yes	○ No (Go to Q688)		
	When yes, what is the pacifier/dummy made of?				
685.	Latex (yellow)	○ Yes	○ No		
686.	Silicon (transparent)	○ Yes	○ No		
687.	Has the pacifier/dummy ever come in contact with an adult's mouth before being given to the child?	○ Yes, regularly	○ Yes, occasionally	○ No	○ Don't know

C. Your child's health

All questions relate to when the child was 13 – 24 months old

688.	Has your child ever had a rash that varies in intensity for at least 6 months? **(Do not count a regular nappy rash)**	○ Yes	○ No
689.	Has your child had a rash or eczema that has lasted for at least 7 days or more? **(Do not count a regular nappy rash)**	○ Yes	○ No
690.	Have any of these rashes at any time affected the fold of the elbows, behind the knees, in front of the ankles, on the cheeks or around the neck, ears or eyes? **(Do not count a regular nappy rash)**	○ Yes	○ No (Go to Q693)
691.	At what age (months) did this rash begin?	☐☐ month/s	
692.	Was this rash itchy?	○ Yes	○ No

693.	Has your child had any of the following skin problems?		
693.	Dry or red patches on the skin	○ Yes	○ No (Go to Q695)
694.	At what age (months) did this begin?	☐☐ month/s	
695.	Swollen lips	○ Yes	○ No (Go to Q697)
696.	At what age (months) did this begin?	☐☐ month/s	
697.	Urticaria/hives	○ Yes	○ No (Go to Q699)

698.	At what age (months) did this begin?	☐☐ month/s

699.	Has your child had an episode of vomiting without any fever?				
699.	Only spit up	○ No	○ Yes, occasionally	○ Yes, often	
700.	Repeated vomiting	○ No	○ Yes, occasionally	○ Yes, often	
701.	Has your child had colic? (sudden continuous crying, bloated stomach, steadily passing gas, cramping and pulling up legs)	○ No (go to Q703)	○ Yes	○ Yes, occasionally	
702.	At what age did it start?	☐☐ month/s			
703.	On average, how many days per week does your child have a bowel movement? (every day=7)	☐ days/week (if <7, go to Q705)			
704.	(only if Q703 is answered with 7) If your child has a bowel movement every day, how many times per day on average?	☐☐ times/day			
705.	Has your child had diarrhoea (at least 3 very loose stools per day) **without any fever**?	○ No (Go to Q707)	○ Yes, only once	○ Yes, occasionally	○ Yes, often
706.	If yes, did it contain blood?	○ No	○ Yes, only once	○ Yes, occasionally	○ Yes, often
707.	Has your child been constipated? (i.e. could not have a bowel movement more than once without aid, such as manipulation, medications, etc.)	○ No	○ Yes, only once	○ Yes, occasionally	○ Yes, often

708.	Has the child had a problem with sneezing or a runny, or blocked nose when s/he did not have a cold or the flu?	○ Yes	○ No (Go to Q722)
709.	Has this nose problem been accompanied by itchy-watery eyes?	○ Yes	○ No
710. – 721.	In which **months** did this nose problem occur? (Please tick any which apply)	Jan ☐ Feb ☐ Mar ☐ Apr ☐ May ☐ Jun ☐ Jul ☐ Aug ☐ Sep ☐ Oct ☐ Nov ☐ Dec ☐	
722.	Has a doctor ever diagnosed the child as having hay fever?	○ Yes	○ No
723.	Has a doctor ever diagnosed the child as having an allergy to house dust?	○ Yes	○ No
724.	Has your child had an allergic reaction when in contact with animals? *(sneezing episodes with multiple sneezes, runny, blocked nose without cold or fever, with watery, red or swollen eyes.)*	○ Yes	○ No (Go to Q732)

725.	Has a doctor ever diagnosed the child as having an allergy to animals?	○ Yes	○ No	
	To which animal(s) has an allergy been diagnosed??			
726.	Dogs	○ Yes	○ No	
727.	Cats	○ Yes	○ No	
728.	Birds	○ Yes	○ No	
729.	Rodents	○ Yes	○ No	
730.	Horses	○ Yes	○ No	
731.	Others (please specify)	_____		
732.	Has the child had an allergic reaction to latex? *(urticaria, especially around mouth, e.g. after use of dummy or blowing up a ballon)*	○ Yes	○ No (Go to Q734)	
733.	Has a doctor diagnosed the child as having an allergy to latex?	○ Yes	○ No	
734.	Has your child had an adverse reaction to bee or wasp stings? *(local itching, urticaria rash or swelling, breathing difficulties or collapse within 2 hours of being stung by bee or wasp)*	○ Yes, just local *(local itching, local urticaria rash or local swelling)*	○ Yes, local and symptomatic *(generalized urticaria, angio-oedema, breathing difficulties or collapse within 2 hours of being stung by bee or wasp)*	○ No (Go to 736)
735.	Has a doctor diagnosed the child as having an allergy to bees or wasps?	○ Yes	○ No	

All questions relate to when the child was 13 – 24 months old

736.	Has your child had wheezing or whistling in the chest <u>when they did not have a cold</u>?	○ Yes	○ No
737.	Has your child's chest sounded wheezy during or after exercise?	○ Yes	○ No
738.	Has your child had a dry cough at night, apart from a cough associated with a cold or chest infection?	○ Yes	○ No
739.	Did a doctor ever diagnose asthma in your child?	○ Yes	○ No
	Have any of the following medications been used?		
740.	bronchodilators	○ Yes	○ No
741.	antihistamines	○ Yes	○ No

742.	**Corticosteroids**		
742a.	oral	○ Yes	○ No
742b.	inhaled	○ Yes	○ No
743.	Does your child have adverse reactions to any foods, such as eczema, breathing problems or gastrointestinal problems?	○ Yes (please complete **Food Allergy Form**)	○ No (Go to Q744)

Food Allergy Form
is a separate Excel form with pull-down menus for each food group and then for each group of symptoms

All questions relate to when the child was 13 – 24 months old

744.	Has the child had allergy testing? *(IgE, SPT, food challenge, etc.)*	○ Yes	○ No (Go to Q749)	○ Don't know (Go to Q749)
	Which tests were positive?			
745.	Serum IgE (blood test)	○ Positive	○ Negative	○ Don't know
746.	Skin Prick Test	○ Positive	○ Negative	○ Don't know
747.	Food challenge	○ Positive	○ Negative	○ Don't know
748.	Other (please specify) 748a_____	○ Positive	○ Negative	○ Don't know

749.	Has your child had injection immunotherapy (desensitisation)?	○ Yes	○ No (Go to Q750)	○ Don't know (Go to Q750)
	For which allergens?	749a.		
		749b.		
		749c.		
750.	Has your child had oral tolerance induction therapy?	○ Yes	○ No (Go to Q751)	○ Don't know (Go to Q751)
	For which allergens?	750a.		
		750b.		
		750c.		

All questions relate to when the child was 13 – 24 months old

751.	How often has your child had any of the following infections?

751.	Upper respiratory infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
752.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q753) ○ Don't know (Go to Q753)
	If yes, please list by which antibiotic(s)	752a. 752b. 752c. 752d. 752e.
753.	Lower respiratory infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
754.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q755) ○ Don't know (Go to Q755)
	If yes, please list by which antibiotic(s)	754a. 754b. 754c. 754d. 754e.
755.	Wheeze in association with an upper respiratory infection (cold)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
756.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q757) ○ Don't know (Go to Q757)
	If yes, please list by which antibiotic(s)	756a. 756b. 756c. 756d. 756e.
757.	Bronchiolitis (bronchitis)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
758.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q759) ○ Don't know (Go to Q759)
	If yes, please list by which antibiotic(s)	758a. 758b.

		758c.
		758d.
		758e.
759.	Middle ear infection	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
760.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q761) ○ Don't know (Go to Q761)
	If yes, please list by which antibiotic(s)	760a.
		760b.
		760c.
		760d.
		760e.
761.	Gastrointestinal illness (diarrhoea/vomiting)	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
762.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q763) ○ Don't know (Go to Q763)
	If yes, please list by which antibiotic(s)	762a.
		762b.
		762c.
		762d.
		762e.
763.	Other infections (list) 763a.----------	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)
764.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q765) ○ Don't know (Go to Q765)
	If yes, please list by which antibiotic(s)	764a.
		764b.
		764c.
		764d.
		764e.
765.	Other infections (list) 765a.----------	○ None/ once ○ Occasionally (once every 3 months) ○ Often (once a month or more)

766.	Was this ever treated with an antibiotic?	○ Yes ○ No (Go to Q767) ○ Don't know (Go to Q767)
	If yes, please list by which antibiotic(s)	766a.
		766b.
		766c.
		766d.
		766e.
767.	Approximately how many times has your child received antibiotics when 13-24 months old?	☐☐ times (if "0" go to Q771)
768.	How old was your child when s/he received the first antibiotic (when 13-24 months old)?	☐☐ months
769.	When did s/he receive the last antibiotic?	☐☐ a. Month ☐☐☐☐ b. Year
770.	What was the name of this antibiotic?	_____
771.	Have you taken your child to a doctor because of any of the following illnesses?	
771.	Weight loss	○ Yes ○ No
772.	Failure to thrive	○ Yes ○ No
773.	Other (not including well-baby checks or immunizations, please specify)	_____
774-775.	What is your child's weight and height?	☐☐.☐ kg Weight ☐☐☐ cm Height At age ☐☐ months
	Has your child received any of the following medications?	
776.	Aspirin	○ Yes ○ No
777.	Paracetemol	○ Yes ○ No
778.	Anti-inflammatories (e.g. Ibuprofen, Nurofen)	○ Yes ○ No
779.	Corticosteroids	
779a.	topical	○ Yes ○ No
779b.	oral	○ Yes ○ No
779c.	inhaled	○ Yes ○ No
780.	Reflux medications	○ Yes ○ No

781.	Cough syrup and/or expectorants	○ Yes ○ No
782.	Other (please specify)	_____
783.	Other (please specify)	_____

784.	Has your child been given any homeopathic or natural supplements, including Chinese medications?	○ Yes ○ No (Go to Q801) ○ Don't know (Go to Q801)
	If yes, give the name of the supplement, the age when it was started, how long it was given and whether the child is still receiving it.	
785.	**Name** of supplement (please specify)	_____
786.	Age when started	☐☐ months
787.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
788.	Still given?	○ Yes ○ No
789.	**Name** of supplement (please specify)	_____
790.	Age when it started	☐☐ months
791.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
792.	Still given?	○ Yes ○ No
793.	**Name** of supplement (please specify)	_____
794.	Age when it started	☐☐ months
795.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days
796.	Still given?	○ Yes ○ No
797.	**Name** of supplement (please specify)	_____
798.	Age when it started	☐☐ months
799.	For how long was it given?	☐☐ a. months or ☐☐ b. weeks or ☐☐ c. days

800.	Still given?	○ Yes	○ No

801.	Has your child received any vaccinations when 13-24 months old?	○ Yes	○ No (go to Q946)			
	What vaccinations has your child received and date(s) received? (from vaccination record)					
802-809.	6-Pack (polio, diphtheria, whooping cough, tetanus, Hepatitis B, H. influenza B)	month year	month year	month year	month year	
810-817.	5-Pack (polio, diphtheria, whooping cough, tetanus, H. influenza B)	month year	month year	month year	month year	
818-825.	Polio	month year	month year	month year	month year	
826-833.	Diphtheria	month year	month year	month year	month year	
834-841.	Whooping cough	month year	month year	month year	month year	
842-849.	Tetanus	month year	month year	month year	month year	
850-857.	Hepatitis B	month year	month year	month year	month year	
858-865.	Hemophilus influenza B	month year	month year	month year	month year	
866-873.	MMR (measles, mumps, rubella)	month year	month year	month year	month year	
874-881.	Measles	month year	month year	month year	month year	
882-889.	Mumps	month year	month year	month year	month year	
890-897.	Rubella (German measles)	month year	month year	month year	month year	
898-905.	Meningitis C	month year	month year	month year	month year	
906-913.	Tuberculosis (BCG)	month year	month year	month year	month year	

914-921.	Pneumococcal (Pneumovax)	month year	month year	month year	month year
922-929.	Chicken pox	month year	month year	month year	month year
930-937.	Other (please specify) 937a_____	month year	month year	month year	month year
938-945.	Other (please specify) 938a_____	month year	month year	month year	month year

946.	What do you use to bathe your child?	○ warm water only ○ baby soap or liquid/wash ○ regular soap or liquid/wash ○ other
947.	Other (please specify)	_____
948.	Do you use any creams, lotions or powders on your child's skin?	○ Yes ○ No (Go to Q 951)
949.	If yes, please specify most commonly used brand / product name(s)	_____
950.	Type	☐ a. Cream/lotion ☐ b. Powder
		_____
	Type	☐ a. Cream/lotion ☐ b. Powder
950c.	How many hours a week does your child watch television or DVDs?	☐☐☐ hours
950d.	How would you describe your child's general physical activity level?	○ Active ○ Normal ○ Sedentary

D. You and your household

All questions relate to when the child was 13 – 24 months old

951.	Has there been any new allergies or allergic symptoms in the child's mother, father or blood-related sibling(s)?	○ Yes (please complete the appropriate **"Changes in Allergy"** Form)	○ No (please ensure all **Baseline Allergy Questionnaires** are completed)
952.	Do you (mother) smoke?	○ Yes, daily ○ Yes, occasionally	○ No

953.	Does anyone else smoke <u>inside</u> your home?	○ Yes, daily ○ Yes, occasionally ○ No
954.	Is your child exposed to tobacco smoke outside the home? (for example at grandparents or other relatives, baby sitter)	○ Yes, daily ○ Yes, occasionally ○ No
955.	Do you work in paid employment at the moment?	○ Yes ○ Yes, but currently on maternity leave (Go to Q957) ○ No (Go to Q957)
956.	How old was your child when you first went back to work?	☐☐ months
957.	Does your child attend day care or a nursery?	○ Yes ○ No (Go to Q988)
958.	How many hours a week on average does your child attend day care or nursery?	☐☐ **hours**
959.	How old was your child when he/she first started day care?	☐☐ months
960.	What type of day care does your child attend?	○ Childminder ○ Nursery/crèche
961.	Approximately how many other children are cared for by the childminder or attend the nursery/crèche?	☐☐ children
962.	Does the childminder OR nursery/crèche have a pet(s)	○ Yes ○ No (Go to Q988)

963-987.	If yes, please choose up to 5 animals from the list, list the number of each and where they are allowed (multiple answers possible)	

	Number of each		Where are they allowed?			
			Where child sleeps	Living room	Kitchen	Only outside the house
Dogs			☐	☐	☐	☐
Cats			☐	☐	☐	☐
Birds			☐	☐	☐	☐
Rodents			☐	☐	☐	☐
Horses			☐	☐	☐	☐
Goats			☐	☐	☐	☐
Cows			☐	☐	☐	☐
Chickens			☐	☐	☐	☐
Pigs			☐	☐	☐	☐
Reptiles			☐	☐	☐	☐
Insects			☐	☐	☐	☐
Fish			☐	☐	☐	☐

All questions relate to when the child was 13 – 24 months old

988.	Have you moved?	○ Yes ○ No (Go to Q996)
989.	What is your new post code?	☐☐☐☐☐☐☐☐
990.	If you don't know your new post code, in what is the name of the suburb or town in which do you now live?	_____
991.	In what type of area do you now live?	○ Urban ○ Rural
992.	What is the approximate population of your city or town?	☐☐ ☐☐☐ ☐☐☐
993.	If you now live in a rural area, do you live on a farm?	○ Yes ○ No
994.	Do you now live on or near a main road where heavy vehicles (trucks, buses) pass by?	○ Yes ○ No
995.	Are there any areas of mould in your flat or house?	○ Yes ○ No ○ Don't know

996.	What main type of flooring is in the room where your child sleeps?	○ Carpet ○ Wooden, laminate, parquet ○ Linoleum or vinyl tiles ○ Ceramic / terracotta tiles or stone ○ Sea-grass or coir-type matting ○ Other
997.	If other, please specify	_____

998.	What kind of mattress does your child sleep on?	○ Raw hair ○ Foam ○ Synthetic (other than foam) ○ Feather ○ Other
999.	If other, please specify	_____
1000.	Does your baby's mattress have a plastic surface or cover?	○ Yes ○ No
1001.	What type of bedding is on your child's bed?	○ blankets ○ Feather or down-filled quilt ○ Synthetic-filled quilt ○ Other (please specify)
1002.	If other, please specify	_____

	If your infant has had more than one type of permanent bed (i.e. a cot or cradle and then a child's crib), please give the information for the previous bed	
998a.	What kind of mattress did your child sleep on?	○ Raw hair ○ Foam ○ Synthetic (other than foam) ○ Feather ○ Other
999a.	If other, please specify	_____
1000a.	Did your baby's mattress have a plastic surface or cover?	○ Yes ○ No
1001a.	What type of bedding was on your child's bed?	○ blankets ○ Feather or down-filled quilt ○ Synthetic-filled quilt ○ Other (please specify)
1002a.	If other, please specify	_____

1003.	Does your baby regularly (at least once a week) share a bed with you?	○ Yes ○ No
1004-1012.	How do you wash your child's clothes and bedding (please check on all that apply)	☐ Reg. Washing soap ☐ Baby (mild) washing soap ☐ Rinse with softener ☐ Rinse with vinegar ☐ Extra rinse ☐ Air-dry clothes ☐ Dry in the dryer ☐ Iron clothes or bedding ☐ Other (please specify)
1013.	If other, please specify	_____

1014.	Do you have any animals?	○ Yes ○ No (Go to Q1040)
1015-1039.	If yes, please choose up to 5 animals from the list, list the number of each and where they are allowed (multiple answers possible)	

	Number of each	Where are they allowed? Where child sleeps	Living room	Kitchen	Only outside the house
Dogs		☐	☐	☐	☐
Cats		☐	☐	☐	☐
Birds		☐	☐	☐	☐
Rodents		☐	☐	☐	☐
Horses		☐	☐	☐	☐
Goats		☐	☐	☐	☐
Cows		☐	☐	☐	☐
Chickens		☐	☐	☐	☐
Pigs		☐	☐	☐	☐
Reptiles		☐	☐	☐	☐
Insects		☐	☐	☐	☐
Fish		☐	☐	☐	☐

1040.	What do you usually use to clean your kitchen work surfaces?	○ Non-bactericidal cleaning product ○ Bactericidal cleaning product ○ None of these ○ Don't know
1041.	What do you usually use to clean the table where the child eats?	○ Antibacterial spray cleaner ○ Soap and water ○ Just water ○ None of these
1042.	How often do you wash your hands after eating peanuts or peanut butter before handling this child?	○ Always ○ Most of the time ○ Sometimes ○ Seldom / never ○ Do not eat peanuts / peanut butter
1043.	How many adults live in the household?	☐☐ (Number)
1044.	How many children live in the household? (including this child)	☐☐ (Number)
1045.	How many bedrooms does your home have, including the child's room and guest room?	○ 1 ○ 2 ○ 3 ○ 4 ○ 5 ○ 6 ○ 7 ○ 8+

Mother's Quality of life

We would like for you to answer a few questions about your current health state. Please indicate which statements best describe your own health state today.

QoL1. Mobility

- I have no problems in walking about ❑
- I have some problems in walking about ❑
- I am confined to bed ❑

QoL2. Self-Care

- I have no problems with self-care ❑
- I have some problems washing or dressing myself ❑
- I am unable to wash or dress myself ❑

QoL3. Usual Activities (*e.g. work, study, housework, family or leisure activities)*

- I have no problems with performing my usual activities ❑
- I have some problems with performing my usual activities ❑
- I am unable to perform my usual activities ❑

QoL4. Pain/Discomfort

- I have no pain or discomfort ❑
- I have moderate pain or discomfort ❑
- I have extreme pain or discomfort ❑

QoL5. Anxiety/Depression

- I am not anxious or depressed ❑
- I am moderately anxious or depressed ❑
- I am extremely anxious or depressed ❑

QoL6. To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion.

Your own health state today

	Date Questionnaire completed	☐☐ Day ☐☐ Month ☐☐☐☐ Year

If any symptoms of an **allergy** were related to intake of particular foods, **please remember** to complete the

Food Allergy Form

If there have been **any changes in allergies** in the mother, father or blood-related sibling, **please remember** to complete the

Change in Allergies Questionnaire

for that person/those persons

Thank mother / father for their participation!

A.5 Thorax Publication

Respiratory research

ORIGINAL ARTICLE

Prevalence estimates and risk factors for early childhood wheeze across Europe: the EuroPrevall birth cohort

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ABSTRACT

Background Preschool wheeze is an important problem worldwide. No comparative population-based studies covering different countries have previously been undertaken.

Objective To assess the prevalence of early childhood wheeze across Europe and evaluate risk factors focusing on food allergy, breast feeding and smoke exposure.

Methods Infants from nine countries were recruited into the EuroPrevall birth cohort. At 12 and 24 months, data on wheeze, allergic signs/symptoms, feeding, smoke exposure, infections and day care attendance were collected using questionnaires. Poisson regression was used to assess risk factors for wheeze.

Results 12 049 infants were recruited. Data from the second year of life were available in 8805 (73.1%). The prevalence of wheeze in the second year of life ranged from <2% in Lodz (Poland) and Vilnius (Lithuania) to 13.1% (95% CI 10.7% to 15.5%) in Southampton (UK) and 17.2% (95% CI 15.0% 19.5%) in Reykjavik (Iceland). In multivariable analysis, frequent lower respiratory tract infections in the first and second years of life (incidence rate ratio (IRR) 1.9 (95% CI 1.3 to 2.6) and 2.5 (95% CI 1.9 to3.4), respectively), postnatal maternal smoking (IRR 1.6, 95% CI 1.1 to 2.4), day care attendance (IRR 1.6, 95% CI 1.1 to 2.5) and male gender (IRR 1.3, 95% CI 1.0 to 1.7) were associated with wheeze. The strength of their association with wheeze differed between countries. Food allergy and breast feeding were not independently associated with wheeze.

Conclusion The prevalence of early childhood wheeze varied considerably across Europe. Lower respiratory tract infections, day care attendance, postnatal smoke exposure and male gender are important risk factors. Further research is needed to identify additional modifiable risk factors that may differ between countries.

Key messages

What is the key question?
- How does the prevalence of early childhood wheeze vary across Europe and what are the key risk factors?

What is the bottom line?
- This study demonstrated that the prevalence of early childhood wheeze varies considerably across Europe and that lower respiratory tract infections, postnatal maternal smoking, day care attendance and male gender are important risk factors.

Why read on?
- This is the first multicentre cohort study to compare the prevalence of and explore risk factors for early childhood wheeze across Europe.

INTRODUCTION

Preschool wheeze affects approximately one-third of children in the first 3 years of life placing a substantial burden on healthcare resources.[1 2] Genetic factors play a role in the aetiology of preschool wheeze and asthma.[3] However, the International Study of Asthma and Allergies in Childhood (ISAAC) and European Community Health Respiratory Survey (ECHRS) recognised that environmental factors are predominantly responsible for geographical variations in the prevalence of asthma. These studies examined international prevalence patterns of asthma symptoms in school-age children and adults, respectively.[4 5] One study has investigated preschool wheeze at age 4 years, but this used data from 10 independent cohorts in eight countries.[6] Further examining variations in prevalence rates of preschool wheeze within a single multicentre cohort may provide new aetiological clues.

Established risk factors for preschool wheeze include male gender, a family history of allergic disease, cigarette smoke exposure, respiratory tract infections and day care attendance.[2 3 7 8] The role of breast feeding in the development of allergic disease and asthma has been extensively investigated with inconclusive findings.[9–11] Several studies have reported that exclusive breast feeding for at least 4 months protects against childhood wheezing.[12 13] However, others have suggested that delaying the introduction of solids may increase the risk.[14 15] Methodological differences may account

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for discrepancies between studies. Therefore, large multicentre studies would help to clarify the role of infant feeding practices in the development of preschool wheeze. Another knowledge gap that needs to be addressed is the relationship between food allergy and preschool wheeze. Numerous studies have shown that food allergy and asthma are closely linked.[16] However, few studies have investigated the relationship between food allergy and preschool wheeze. In particular, studies using double-blind placebo-controlled food challenges (DBPCFC) are lacking.

This study aimed to determine the prevalence of wheeze in the first 2 years of life across Europe. It further aimed to evaluate risk factors for wheeze, focusing on food allergy, infant feeding and cigarette smoke exposure. We hypothesised that early-onset food allergy increases the risk of early childhood wheeze and that longer duration of breast feeding and increased overlap between breast feeding and solids are protective. Many other potential influences were considered, including exposure to cigarette smoke during pregnancy and infancy, birth weight, birth length and gestation.

METHODS

Study design

The EuroPrevall birth cohort was established between 2005 and 2010. The methodology and baseline characteristics have previously been reported.[17 18]

Evaluation began at birth with follow-up of participants at 12 and 24 months using standardised questionnaires based on those used in previous epidemiological studies such as ISAAC. Questionnaires were administered via phone or in person by trained personnel. Additional assessments, including skin prick testing, measurement of specific IgE with or without a DBPCFC were performed according to a standardised protocol whenever parents reported symptoms suggestive of food allergy in their children.[17 18]

Study population

Families were recruited antenatally and postnatally from nine study centres: Reykjavik (Iceland), Southampton (UK), Amsterdam (The Netherlands), Berlin (Germany), Lodz (Poland), Vilnius (Lithuania), Madrid (Spain), Milan (Italy) and Athens (Greece).[17]

Inclusion criteria were a gestational age of at least 34 weeks and a good condition at birth (Apgar score of at least 7 at 5 minutes). Families unable to give informed consent and infants participating in other studies examining allergic disease were excluded. Written informed consent was obtained from all parents.[17 18]

Follow-up questionnaires were not always completed at the intended ages of 12 months and 24 months. Participants were included in this analysis if data were collected between the ages of 6 months and less than 18 months (1-year data) or 18 months and 30 months of age (2-year data).

Exposures

At recruitment, data were collected on birth details, maternal diet, family history, maternal education (as a marker of socioeconomic status) and environmental exposures, including cigarette smoke and pet ownership.

The 12-month and 24-month questionnaires included an extensive list of foods found in children's diets. Parents were asked if their child had tried each food and if so, when they first tried it. Parents were also asked if their child had ever been breast fed and, if so, for how long. Using these data, the age of each child when solids were first introduced and the overlap (in months) between breast feeding and solids was determined.

A number of dichotomous smoking variables were generated using data collected at baseline and 12 months. These are described in *appendix A*, along with other baseline and follow-up variables, which were assessed as potential risk factors. These included day care attendance, respiratory tract infections and eczema.

Food allergy was defined as a positive DBPCFC or clear history of anaphylaxis to any food in the first 24 months of life. Children with food allergy were subdivided into those with IgE-mediated and non-IgE-mediated food allergy. IgE-mediated food allergy was defined as food allergy with a positive skin prick test (≥3 mm weal) or positive specific IgE (≥0.35 kU/L) at any time during follow-up.

Outcomes

Wheeze in the second year of life was the primary outcome for this analysis. Questions relating to wheeze included: 'In the last 12 months, has your child had wheezing or whistling in the chest?' (12-month questionnaire) and 'Between the ages of 13–24 months, has your child had wheezing or whistling in the chest when they did not have a cold?' (24-month questionnaire). Children were defined as having wheeze in the second year of life if parents answered yes to either of these questions within the specified time range for 2-year data. A secondary analysis comparing those with recurrent wheeze (wheeze in both the first and second years of life) to a never wheezed group was undertaken.

Statistical analysis

Statistical analysis was undertaken using STATA SE V.13. The baseline characteristics and exposures of participants were described for the whole cohort, separately for each centre and those with and without wheeze in the second year of life. No data were imputed. Differences between centres were examined, appropriate descriptive statistics were undertaken and differences were compared using χ^2 (dichotomous/categorical variables), one-way analysis of variance (continuous, normally distributed variables) or Kruskal-Wallis (continuous, non-normally distributed variables).

Poisson regression was used to identify risk factors for wheeze in the second year of life. Variables associated with wheeze (P value <0.1), food allergy and variables related to feeding and cigarette smoke exposure were entered into a multivariable model. A dummy variable for study centre (using Reykjavik as the baseline centre) was included in the model to account for heterogeneity between centres. Variables were not included if they were explained by combinations of others, for example, allergic disease and allergic rhinitis. Three alternative multivariable models were generated in a sensitivity analysis. Sensitivity model one was derived by applying backward deletion to the primary model; that is, variables were sequentially removed (starting with the variable with the weakest association with wheeze) until only those with a P value ≤0.05 remained in the model. Sensitivity models two and three were similar to the primary model and sensitivity model one, respectively, but did not include study centre. Likelihood ratio tests were used to test the goodness of fit of the multivariable models. Significant associations from the primary model were entered into a separate multivariable model to examine their importance in individual centres.

RESULTS

Participants

The EuroPrevall cohort included 12 049 infants. A total of 6189 (51.4%) were male. The baseline characteristics of participants varied considerably between centres (table 1 and online supplementary table S1).[18] After excluding participants followed up outside the specified age ranges for 1-year and 2-year data, follow-up data were available in 8174 infants (67.8%) at 1 year and in 8805 infants (73.1%) at 2 years (figure 1). Follow-up rates varied between centres (online supplementary table S2). The baseline characteristics of those with 2-year data were similar to those without (online supplementary table S3).

Prevalence of wheeze and potential risk factors

The prevalence of wheeze in the second year of life across all centres was 7.8%, ranging from 1.7% in Lodz to 17.2% in Reykjavik (table 2 and figure 2). Large differences in the prevalence of recurrent wheeze were also seen, ranging from 0.3% in Vilnius to 10% in Reykjavik (table 2).

Among children included in this analysis, the prevalence of food allergy ranged from 0.1% in Athens to 3% in Southampton. The majority of cases of food allergy were IgE mediated (table 3). The mean duration of breast feeding was 6.1 months overall, ranging from 4.3 months in Athens to 8.5 months in Reykjavik. The age at introduction of solids was similar across Europe ranging from 5.0 months to 5.7 months. Maternal smoking habits (during pregnancy and postnatally) also varied considerably between centres (table 3).

Association of risk factors with wheeze in the second year of life

Food allergy

21.5% of infants with food allergy had wheeze in the second year of life compared with 7.6% of infants without. Although food allergy was associated with wheeze in the second year of life in univariate analysis (raw incidence rate ratio (IRR) 2.84, 95% CI 1.92 to 4.20, P<0.001) (table 4a), this association was not consistent across centres (online supplementary table S6) and was not significant after adjusting for potential confounders (adjusted IRR 1.26, 95% CI 0.55 to 2.91, P 0.589) (table 4a).

Feeding practices

In univariate analysis, breast feeding, longer duration of breast feeding and increased overlap of breast feeding/solids were associated with a lower prevalence of wheeze in some centres (online supplementary table S6). In the primary model, however, none of these factors were statistically significant (table 4a). Increased overlap of breast feeding/solids showed a small protective effect against wheeze (adjusted IRR 0.95, 95% CI 0.90 to 1.00) in sensitivity model one (table 4a).

Smoke exposure

Univariate analysis suggested that any maternal smoking increases the risk of wheeze in the second year of life (raw IRR 1.29, 95% CI 1.11 to 1.50, P 0.001), while having other household smokers decreases the risk of wheeze (raw IRR 0.81, 95% CI 0.66 to 0.98, P 0.033). However, neither of these factors were independently associated with wheeze in the second year of life (table 4a). Maternal smoking at 1-year follow-up was a statistically significant risk factor for wheeze in multivariable analysis (adjusted IRR 1.62, 95% CI 1.09 to 2.42, P 0.017) (table 4a).

Other potential risk factors

Other factors associated with wheeze in the second year of life in univariate analysis included male gender, higher birth weight, eczema, a family history of allergic disease, day care attendance and frequent (≥quarterly) respiratory tract infections. Dog ownership and longer birth length were associated with a lower prevalence of wheeze (table 4b). In multivariable analysis, only frequent lower respiratory tract infections (LRTIs) in the second year of life (adjusted IRR 2.50, 95% CI 1.83 to 3.41, P<0.001), frequent LRTIs in the first year of life (adjusted IRR 1.87, 95% CI 1.33 to 2.64, P<0.001), day care attendance (adjusted IRR 1.63, 95% CI 1.08 to 2.45, P 0.020), maternal smoking at 1-year follow-up (adjusted IRR 1.62, 95% CI 1.09 to 2.42, P 0.017) and male gender (adjusted IRR 1.33, 95% CI 1.03 to 1.70, P 0.027) were statistically significant risk factors for wheeze (tables 4a and 4b). Male gender and frequent LRTIs were also risk factors for recurrent wheeze, along with maternal allergy and paternal asthma (online supplementary table S5).

Alternative models

When 'study centre' was removed from the primary model, paternal allergy (adjusted IRR 1.36, 95% CI 1.01 to 1.83, P 0.004), frequent upper respiratory tract infections (URTIs) in the first year of life (adjusted IRR 1.55, 95% CI 1.09 to 2.19, P 0.014) and frequent URTIs in the second year of life (adjusted IRR 1.62, 95% CI 1.11 to 2.36, P 0.012) were identified as risk factors for wheeze (online supplementary table S4).

The association between the risk factors identified by the primary model and wheeze in the second year of life varied between centres (table 5). In Southampton, for example, only male gender (IRR 1.66, 95% CI 1.00 to 2.76, P 0.050) and maternal smoking at 1-year follow-up (adjusted IRR 2.72, 95% CI 1.29 to 5.7, P 0.009) were statistically significant risk factors for wheeze.

DISCUSSION

This study has demonstrated that the prevalence of parent-reported wheeze in the first 2 years of life varies considerably across Europe with a broadly northwestern to southeastern gradient. The strongest risk factors for wheeze were lower respiratory tract infections, day care attendance, postnatal maternal smoking and male gender. Many other potential risk/protective factors including food allergy and breast feeding were evaluated. None of these had a significant influence on the prevalence of wheeze in the second year of life.

Wheeze prevalence

The prevalence of wheeze in the second year of life across all centres was 7.8%. For the Netherlands, the estimated prevalence of wheeze (11%) was lower than in the Generation R study. This estimated the prevalence of wheeze in Rotterdam at 2 years to be 20%.[19] Our estimates for Southampton (13%), Berlin (12%) and Amsterdam (11%) were, however, similar to the 12% estimate in the Pollution and Asthma Risk: an Infant Study (PARIS) birth cohort.[8] Our study is the first multicentre cohort study to assess the prevalence of early childhood wheeze across Europe. The prevalence of wheeze in the second year of life was nearly 10-times higher in Reykjavik and Southampton than in Lodz and Vilnius. In keeping with our findings, the ISAAC study and ECRHS reported a high prevalence of wheeze in Western Europe with lower prevalences in Eastern and Southern Europe.[20] Countries represented in EuroPrevall, ISAAC and ECRHS included the UK, Germany, Italy, Spain and

Appendix A

Table 1 Key baseline characteristics of the EuroPrevall cohort by centre

	All centres (n=12 049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
Basic demographics and birth details										
Male gender (%)	51.4	51.2	51.2	52.7	51.7	51.7	51.2	50.7	50.1	52.6
Gestation (weeks), median (range)†	39 (34–44)	40 (34–44)	40 (34–43)	40 (34–43)	39 (34–43)	39 (34–42)	39 (34–42)	39 (34–43)	39 (34–43)	40 (34–44)
Birth weight (kg), mean (SD)‡	3.40 (0.51)	3.76 (0.51)	3.46 (0.52)	3.48 (0.51)	3.41 (0.49)	3.28 (0.50)	3.52 (0.44)	3.25 (0.43)	3.27 (0.51)	3.20 (0.46)
Caesarean section (%)*	24.0	12.8	30.8	11.0	31.1	37.5	15.6	2.5	30.8	44.2
Caucasian mother (%)*	93.3	99.2	95.9	72.2	93.4	99.9	99.9	84.5	89.9	99.2
Caucasian father (%)*	92.7	98.4	97.0	69.7	90.0	99.3	99.5	84.8	90.4	99.4
Maternal age (years), mean (SD)†	30.7 (5.21)	30.1 (4.81)	31.8 (5.18)	29.9 (4.82)	31.4 (5.41)	28.8 (4.43)	28.2 (5.20)	31.4 (5.10)	33.6 (4.66)	30.9 (4.86)
Paternal age (years), mean (SD)†	33.3 (6.10)	32.2 (5.50)	34.1 (5.67)	33.0 (6.07)	34.6 (6.61)	31.1 (5.40)	30.9 (6.25)	33.7 (5.65)	36.1 (5.75)	34.8 (5.47)
Familial allergic disease										
Maternal self-reported, doctor-diagnosed allergic disease (%)										
Any*	26.3	44.5	51.4	36.5	35.3	9.7	5.9	24.8	23.7	14.0
Asthma*	9.4	17.2	22.8	13.3	10.8	3.2	1.5	6.2	8.7	5.2
Paternal self-reported, doctor-diagnosed allergic disease (%)										
Any*	21.0	32.1	41.0	30.8	29.3	8.7	2.8	19.4	21.4	11.7
Asthma*	7.2	9.9	19.4	9.8	8.7	2.1	0.8	5.2	8.7	3.7
Living environment										
Rural housing (%)*	16.1	7.2	76.1	0.6	2.2	22.4	13.6	12.3	8.6	7.9
Mould in house (%)*	9.9	7.6	10.7	17.5	11.4	2.0	10.2	4.6	9.3	21.5
Pets (%)										
Any*	35.5	28.0	50.2	54.5	33.1	46.3	43.8	23.5	24.4	19.5
Cat*	15.1	11.3	28.8	26.8	15.0	15.1	22.0	5.4	10.5	3.7
Dog*	16.0	9.3	18.1	14.1	9.0	34.8	21.7	13.5	10.8	10.2
Maternal education										
Only basic education completed (%)*	18.2	15.1	10.8	11.8	10.8	27.2	19.1	24.9	12.8	31.0

Study centre (n)=total number of infants recruited.
Allergic disease was defined as asthma, allergic rhinitis and/or eczema.
Rural housing was defined as living in a village or the countryside and urban living was defined as living in a town or city.
Basic education was defined as completing 10 years in school.
*P<0.05 using χ^2 to test differences between centres.
†P<0.05 using Kruskal-Wallis to test differences between centres.
‡P<0.05 using one-way analysis of variance to test differences between centres.

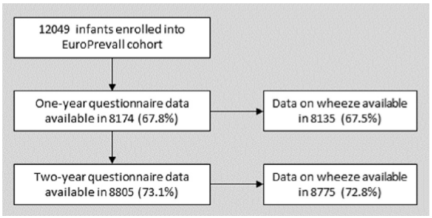

Figure 1 EuroPrevall participants included in this analysis.

Greece. Across these countries, the prevalence of self-reported wheeze/diagnosed current asthma (ECHRS) was highest in the UK and lowest in Greece in all three studies.[21 22] This suggests that common factors are driving early childhood wheeze and asthma in later life.

Risk factors for early childhood wheeze

The allergic march describes the progression from eczema in early childhood to asthma and allergic rhinitis later on.[23 24] The role of food allergy in this is unclear[24] though food allergy is known to be associated with asthma at school age.[25] A substantial number of children who wheeze in infancy later develop asthma.[2 26] Therefore, we hypothesised that food allergy is a risk factor for wheeze in the first 2 years of life. Although food allergy was significant in univariate analysis, when potential confounders were considered, no association with wheeze was seen. According to the primary model, this was also true for eczema. In keeping with our results, the Urban Environment and Childhood Asthma study reported no association between food allergy (diagnosed according to IgE levels ≥0.35 and a history suggestive of food allergy) and wheeze in the first 2 years life,[27] and in the Tuscon Children's Respiratory Study, eczema was not a risk factor for transient early wheezing.[2] A likely explanation for these findings is that early childhood wheeze is predominantly driven by respiratory tract infections rather than atopy. Indeed, LRTIs were associated with wheeze in all of the multivariable models that we tested.

Day care attendance increases exposure to respiratory tract infections.[28] Therefore, as expected, this was associated with wheeze in the second year of life. The PARIS and Prevention and Incidence of Asthma and Mite Allergy (PIAMA) birth cohorts also found that early day care attendance is associated with increased wheeze before the age of 4 years.[8 28] Several studies have, however, reported a protective effect of day care attendance on asthma at school age,[29–31] reflecting the fact that preschool wheeze and asthma are different entities. In a post hoc analysis, we investigated whether the relationship between day care and wheeze is influenced by age at entry to day care or the number of hours spent in day care in the first year of life. In univariate analysis, entering day care later was associated with a lower risk of wheeze (IRR 0.98 (per month increase), P 0.26, 95%CI 0.97 to 1.00). However, neither age at entry to day care nor the number of hours spent in day care in the first year of life was significantly associated with wheeze in the second year of life when included in the primary model.

Of the smoking variables evaluated, only maternal smoking at 1-year follow-up was independently associated with wheeze in the second year of life. Previous studies have demonstrated that maternal smoking during pregnancy is an independent risk

Table 2 Prevalence of wheeze in the first 2 years of life by centre

	All centres (n=12 049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
Wheeze in the first year of life, % (95% CI)	13.5 (12.7 to 14.2)	33.9 (31.0 to 36.9)	31.6 (27.9 to 35.2)	26.1 (23.2 to 29.1)	9.1 (7.6 to 10.6)	11.4 (9.6 to 13.2)	0.5 (0.1 to 0.9)	4.1 (2.8 to5.3)	**	2.0 (1.0 to 2.9)
Wheeze in the second year of life, % (95% CI)	7.8 (7.2 to 8.3)	17.2 (15.0 to 19.5)	13.1 (10.7 to 15.5)	10.8 (8.3 to 13.2)	11.8 (10.0 to 13.5)	1.7 (1.0 to 2.4)	1.9 (1.1 to 2.7)	3.0 (1.9 to 4.1)	9.5 (7.6 to 11.4)	2.8 (1.6 to 3.9)
Recurrent wheeze (wheeze in the first and second years of life), % (95% CI)	3.1 (2.7 to 3.5)	10.0 (7.9 to 12.0)	7.9 (5.6 to 10.3)	6.4 (4.4 to 8.4)	1.8 (1.1 to 2.6)	0.8 (0.2 to 1.3)	0.3 (0.0 to 0.6)	2.0 (1.0 to 2.9)	**	0.4 (0.0 to 0.8)

Study centre (n)=total number of infants recruited.
P<0.05 for all variables using χ^2 to test differences between centres.
**For Milan, the prevalences of wheeze in the first year of life and recurrent wheeze are not specified because 1-year data were not available for most participants.

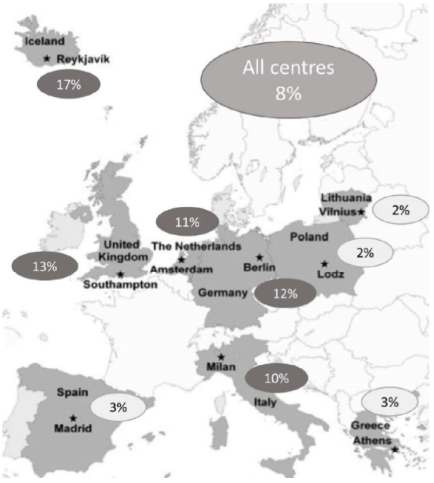

Figure 2 Map showing study centres and the prevalence of wheeze in the second year of life in each centre (adapted from Keil *et al*).[17]

factor for wheeze in infancy.[19 32–35] In this analysis, the number of cigarettes smoked during pregnancy and the timing of smoke exposure was not considered. This may account for the discrepancy between our findings and those of other studies. Another unexpected finding was that other household smokers were associated with a lower risk of wheeze in univariate analysis. However, once potential confounders were considered, there was a non-significant trend for other household smokers to increase the risk of wheeze.

Given breast milk contains antiviral antibodies, a protective effect on early childhood wheeze is plausible.[11] It has previously been concluded that exclusive breast feeding for at least 4 months reduces the risk of recurrent wheeze in childhood.[10 11] However, we found no association between breast feeding or breast feeding duration and wheeze in the second year of life. This may be due to the fact that the mean duration of breast feeding was more than 4 months in all centres, making it more difficult to demonstrate a protective effect. Nevertheless, when the relationship between breast feeding duration and wheeze was analysed using a categorical variable based on quartiles, the same effect was seen. Increased overlap between breast feeding and solids showed a small protective effect against wheeze in sensitivity model one. Grimshaw *et al* previously demonstrated that concurrent feeding with breast and cow's milk reduces the risk of food allergy in infancy,[36] while Snijders *et al* reported that delaying the introduction of cow's milk and solids increases the risk of eczema and wheeze, respectively.[15] A potential explanation for these findings is that breast milk only has beneficial immunomodulatory effects when the immune system is exposed to other dietary proteins.[36] Further research is needed to establish whether maximising the duration of overlap between breast and complementary feeding could help to prevent childhood wheeze.

Given that heterogeneity between centres in terms of both baseline factors and potential risk factors for wheeze was observed, the primary model and sensitivity model one were adjusted for study centre. Study centre was significant suggesting that unmeasured factors are operating in individual centres. Furthermore, when significant associations from the primary model were investigated by centre, their importance varied. Confounding between centres and these variables may partially explain differences between the unadjusted and adjusted model estimates. Climatic differences may also be important. For example, the colder climate in northern Europe may predispose to more frequent respiratory tract infections and hence wheeze.

Strengths and limitations

A potential limitation of any longitudinal study is loss to follow-up. As outlined in online supplementary table S1, follow-up varied between centres. The number of infants from Milan with 1-year data was especially low because the dates on which most participants' 12-month questionnaires were completed were not available. This made it impossible to determine the age of these infants at the time of data collection. Overall, however, '2 year' data were available in over 70% of participants, and the baseline characteristics of those with and without '2 year' data were similar.

At four centres, anonymous data on family history and education were collected from 2320 parents who declined participation. Parents who agreed to participate in the study had a higher level of education and were more likely to have a history of allergic diseases.[18 37] Given that paternal allergic disease and maternal asthma were associated with wheeze in some models, the prevalence of wheeze among infants in the EuroPrevall cohort may be higher than in the general population.

Another important consideration is that wheeze prevalence estimates were dependent on parents' understanding of the term wheeze.[38] Similarly, some children with viral rashes may have been wrongly classified as having eczema. This is suggested by the fact that the prevalence of eczema in individual centres was largely concordant with the prevalence of URTIs. To minimise the potential for misunderstanding, study questionnaires were translated from English into different languages and verified with back translation. Furthermore, they were based on the widely used ISAAC questionnaires, which have been validated in many languages for assessing wheeze and asthma in school-age children.[4 39] Recall bias is possible given that some questionnaires were completed 6 months after the period for which they were intended to capture data.

Major strengths of this study are its size (allowing us to adjust for multiple confounders) and the fact that diagnoses of food allergy were confirmed by double-blind, placebo-controlled challenges.

CONCLUSION

This birth cohort study provides unique data on the prevalence of and risk factors for early childhood wheeze across Europe. Early onset food allergy and infant feeding practices were not associated with wheeze in second year of life. In keeping with previous studies, lower respiratory tract infections, day care attendance, maternal smoking in infancy and male gender were identified as important risk factors. The relationship between these and wheeze differed between centres, suggesting that additional risk factors may be operating in individual countries. Further research is needed to identify these. Meanwhile, preventing respiratory tract infections and minimising postnatal

Table 3 Potential risk factors for wheeze by centre

	All centres (n=12 049)	Reykjavik (n=1341)	Southampton (n=1140)	Amsterdam (n=976)	Berlin (n=1570)	Lodz (n=1513)	Vilnius (n=1556)	Madrid (n=1387)	Milan (n=1486)	Athens (n=1080)
Food allergy										
Any food allergy diagnosed in first 2 years of life (%)*	1.3	2.1	3.0	2.3	1.0	1.2	0.5	1.5	0.8	0.1
IgE-mediated food allergy %*	1.1	1.9	1.9	1.2	1.0	1.1	0.4	1.5	0.8	0.1
Feeding										
Ever breast fed (%)*	90.8	98.4	89.8	81.3	95.8	90.5	94.9	86.1	91.0	83.1
Duration of breast feeding (months), mean (SD)†	6.1 (3.90)	8.5 (3.46)	5.4 (4.14)	4.6 (3.41)	6.4 (3.60)	6.1 (4.44)	5.7 (3.94)	5.3 (2.98)	7.1 (3.76)	4.3 (3.28)
Age at introduction of solids (months), mean (SD)†	5.3 (1.61)	5.3 (1.20)	5.1 (1.36)	5.2 (1.47)	5.5 (1.57)	5.0 (1.27)	5.7 (2.54)	5.1 (1.39)	5.6 (1.48)	5.3 (1.44)
Overlap of breast feeding/solids (months), median (range)‡	0 (0–25.8)	3.2 (0–18.4)	0 (0–23.5)	0 (0–15.4)	1.1 (0–13.3)	0 (0–15.3)	0.1 (0–11.4)	0 (0–11.3)	2.1 (0–11.6)	0 (0–25.8)
Smoke exposure										
Mother ever smoked (%)*	41.7	38.4	41.8	42.9	52.7	34.0	40.6	39.0	40.0	46.4
Smoking at any time during pregnancy (%)*	9.6	7.7	6.7	10.5	10.3	8.8	7.5	10.9	7.8	18.1
Mother smoking at 1-year follow-up (%)*	15.9	10.3	6.3	15.2	16.9	14.4	8.0	23.0	§	33.3
Other smokers in household (%)*	22.9	3.0	17.2	20.2	10.1	31.7	42.9	17.8	27.7	33.4
Day care attendance										
Day care in first year of life (%)*	32.2	61.3	50.0	72.7	38.2	6.5	2.0	40.0	§	3.3
Day care in second year of life (%)*	55.3	93.7	58.6	83.4	81.2	18.6	27.2	63.6	62.5	17.6
Day care at any time in first 2 years of life (%)*	63.7	97.0	73.7	90.7	82.9	22.8	30.6	68.0	§	18.9
Respiratory tract infections										
Frequent URTIs (≥quarterly) in first year of life (%)*	55.5	91.2	77.9	80.3	80.3	54.4	4.0	18.6	§	46.3
Frequent URTIs (≥quarterly) in second year of life (%)*	56.7	91.6	65.1	81.0	90.2	47.8	3.4	22.2	68.8	59.2
Frequent LRTIs (≥quarterly) in first year of life (%)*	5.4	20.9	5.5	1.3	4.6	3.4	0.3	6.0	§	0.8
Frequent LRTIs (≥quarterly) in second year of life (%)*	9.0	23.2	5.8	1.1	6.0	1.0	1.1	5.8	28.6	23.3
Allergic disease										
Eczema in first 2 years of life (%)*	34.7	53.0	56.0	46.1	37.4	33.1	4.9	26.7	§	18.7

Study centre (n)=total number of infants recruited.
*P<0.05 using one-way analysis of variance to test differences between centres.
†P<0.05 using Kruskal-Wallis to test differences between centres.
‡P<0.05 using χ^2 to test differences between centres.
§For Milan, no 1-year outcomes or variables dependent on these are specified because 1-year data were not available for most participants.
LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Table 4a Association of risk factors with wheeze in the second year of life: food allergy, feeding, smoke exposure, day care attendance, respiratory tract infections, eczema and study centre

	Unexposed % (n/N)	Exposed % (n/N)	Wheeze in unexposed % (n/N)	Wheeze in exposed % (n/N)	Unadjusted IRR (95% CI) (P value)	Primary model – Adjusted IRR (95% CI) (P value) (n=3612)	Sensitivity model 1 – Adjusted IRR (95% CI) (P value) (n=4227)
Food allergy							
Food allergy diagnosed in first 2 years of life (yes vs no)	98.6 (8654/8775)	1.4 (121/8775)	7.6 (655/8564)	21.5 (26/121)	2.84 (1.92 to 4.20) (<0.001)	1.26 (0.55 to 2.91) (0.589)	
Feeding							
Ever breast fed (yes vs no)	8.8 (731/7607)	91.2 (7607/8338)	6.0 (44/731)	7.9 (597/7607)	1.30 (0.96 to 1.77) (0.089)	0.67 (0.14 to 3.18) (0.615)	
Duration of breast feeding (per month increase)					1.00 (0.98 to 1.02) (0.918)	1.02 (0.92 to 1.12) (0.729)	
Age at introduction of solids (per month increase)					0.98 (0.93 to 1.03) (0.384)	0.94 (0.83 to 1.08) (0.410)	
Overlap of breast feeding/solids (per month increase)					0.99 (0.96 to 1.03) (0.709)	0.94 (0.82 to 1.07) (0.320)	0.95 (0.90 to 1.00) (0.044)
Smoke exposure							
Mother ever smoked (yes vs no)	59.8 (5243/8774)	40.2 (3531/8774)	7.0 (365/5243)	9.0 (316/3531)	1.29 (1.11 to 1.50) (0.001)	1.06 (0.81 to 1.40) (0.673)	
Smoking at any time during pregnancy (yes vs no)	91.8 (7843/8544)	8.2 (701/8544)	7.7 (601/7843)	9.6 (67/701)	1.25 (0.97 to 1.61) (0.086)	0.66 (0.40 to 1.10) (0.112)	
Mother smoking at 1 year follow-up (yes vs no)	84.6 (5894/6971)	15.5 (1077/6971)	6.9 (404/5894)	7.9 (85/1077)	1.15 (0.91 to 1.45) (0.237)	1.62 (1.09 to 2.42) (0.017)	1.45 (1.09 to 1.92) (0.011)
Other smokers in household (yes vs no)	79.1 (6936/8774)	21.0 (1838/8774)	8.1 (561/6936)	6.5 (120/1838)	0.81 (0.66 to 0.98) (0.033)	1.25 (0.85 to 1.85) (0.261)	
Day care attendance							
Day care in first year of life (yes vs no)	69.1 (4780/6922)	30.9 (2142/6922)	5.0 (239/4780)	11.5 (247/2142)	2.31 (1.93 to 2.76) (<0.001)		
Day care in second year of life (yes vs no)	44.7 (3908/8739)	55.3 (4831/8739)	4.0 (154/3891)	10.8 (522/4820)	2.74 (2.29 to 3.28) (<0.001)		
Day care at any time in first 2 years of life (yes vs no)	38.3 (3051/7966)	61.7 (4915/7966)	3.1 (93/3051)	10.7 (526/4915)	3.51 (2.82 to 4.38) (<0.001)	1.63 (1.08 to 2.45) (0.020)	1.70 (1.18 to 2.45) (0.004)
Respiratory tract infections							
URTIs in first year of life (≥quarterly vs ≤one)	45.2 (3142/6956)	54.8 (3814/6956)	3.3 (102/3142)	10.2 (388/3814)	3.13 (2.52 to 3.90) (<0.001)	1.08 (0.75 to 1.56) (0.672)	
URTIs in second year of life (≥quarterly vs ≤one)	42.1 (3626/8604)	57.9 (4978/8604)	3.8 (136/3626)	10.8 (537/4978)	2.88 (2.38 to 3.47) (<0.001)	1.08 (0.72 to 1.62) (0.704)	
LRTIs in first year of life (≥quarterly vs ≤one)	94.7 (6524/6886)	5.3 (362/6886)	6.2 (406/6524)	22.4 (81/362)	3.60 (2.83 to 4.56) (<0.001)	1.87 (1.33 to 2.64) (<0.001)	1.72 (1.25–2.36) (0.001)
LRTIs in second year of life (≥quarterly vs ≤one)	91.0 7252/7967	9.0 715/7967	6.6 (479/7252)	24.9 (178/715)	3.77 (3.17 to 4.48) (<0.001)	2.50 (1.83 to 3.41) (<0.001)	2.36 (1.76–3.17) (<0.001)

Continued

Table 4a Continued

	Unexposed % (n/N)	Exposed % (n/N)	Wheeze in unexposed % (n/N)	Wheeze in exposed % (n/N)	Unadjusted IRR (95% CI) (P value)	Primary model – Adjusted IRR (95% CI) (P value) (n=3612)	Sensitivity model 1 – Adjusted IRR (95% CI) (P value) (n=4227)
Allergic disease							
Eczema in first2 years of life (yes vs no)	67.7 (4870/7198)	32.3 (2328/7198)	5.1 (247/4870)	12.2 (284/2328)	2.41 (2.03 to 2.85) (<0.001)	1.20 (0.93 to 1.55) (0.158)	1.35 (1.08–1.69) (0.009)
Study centre							
Reykjavik	87.8 (7700/8775)	1075/8775 (12.3)	6.4 (496/7700)	17.2 (185/1075)	Baseline comparator	Baseline comparator	Baseline comparator
Southampton	91.3 (8011/8775)	8.7 (764/8775)	7.3 (581/8011)	13.1 (100/764)	0.76 (0.60 to 0.97) (0.027)	1.04 (0.65 to 1.68) (0.866)	1.09 (0.74–1.60) (0.668)
Amsterdam	92.9 (8153/8775)	7.1 (622/8775)	7.5 (614/8153)	10.8 (67/622)	0.63 (0.47 to 0.83) (<0.001)	0.72 (0.36 to 1.41) (0.335)	0.82 (0.54–1.24) (0.351)
Berlin	85.3 (7482/8775)	14.7 (1293/8775)	7.1 (529/7482)	11.8 (152/1293)	0.68 (0.55 to 0.85) (<0.001)	0.98 (0.69 to 1.39) (0.912)	0.99 (0.73–1.35) (0.957)
Lodz	85.7 (7520/8775)	14.3 (1255/8775)	8.8 (660/7520)	1.7 (21/1255)	0.10 (0.06 to 0.15) (<0.001)	0.18 (0.08 to 0.42) (<0.001)	0.17 (0.08–0.36) (<0.001)
Vilnius	86.7 (7611/8775)	13.3 (1164/8775)	8.7 (659/7611)	1.9 (22/1164)	0.11 (0.07 to 0.17) (<0.001)	0.34 (0.15 to 0.78) (0.010)	0.33 (0.18–0.60) (<0.001)
Madrid	89.6 (7865/8775)	10.4 (910/8775)	8.3 (654/7865)	3.0 (27/910)	0.17 (0.13 to 0.26) (<0.001)	0.18 (0.08 to 0.39) (<0.001)	0.23 (0.13–0.41) (<0.001)
Milan	89.8 (7878/8775)	10.2 (897/8775)	7.6 (596/7878)	9.5 (85/897)	0.55 (0.43 to 0.71) (<0.001)	1.33 (0.17 to 10.20) (0.781)	2.67 (0.65–10.97) (0.172)
Athens	90.9 (7980/8775)	9.1 (795/8775)	8.3 (659/7980)	2.8 (22/795)	0.16 (0.10 to 0.25) (<0.001)	0.34 (0.05 to 2.61) (0.302)	0.33 (0.04–2.40) (0.272)

Primary model: includes all variables with P<0.1 in univariate analysis (gender, gestation, birth weight, birth length, ethnicity of father, maternal allergy, maternal asthma, paternal allergy, paternal asthma, mould in house, dog ownership, day care attendance, respiratory tract infections and eczema), plus food allergy, variables related to feeding and smoke exposure and study centre.
Sensitivity model 1: this was generated by applying backward deletion to the primary model. It includes overlap of breast feeding/solids, mother smoking at 1 - year follow-up , gender, day care attendance, LRTIs , eczema and study centre.
IRR, incidence rate ratio; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Table 4b Association of risk factors with wheeze in the second year of life: demographics, birth details, familial allergic disease, living environment and maternal education

	Unexposed % (n/N)	Exposed % (n/N)	Wheeze in unexposed % (n/N)	Wheeze in exposed % (n/N)	Unadjusted IRR (95% CI) (P value)	Primary model –adjusted IRR (95% CI) (P value) (n=3612)	Sensitivity model 1 – adjusted IRR (95% CI) (P value) (n=4227)
Basic demographics and birth details							
Male gender (vs female)	48.6 (4263/8774)	51.4 (4511/8774)	6.6 (280/4263)	8.9 (401/4511)	1.35 (1.16 to 1.58) (<0.001)	1.33 (1.03 to 1.70) (0.027)	1.32 (1.06 to 1.64) (0.014)
Gestation (per week increase)					1.04 (0.99 to 1.10) (0.105)	1.02 (0.93 to 1.12) (0.700)	
Birth weight (per kg increase)					1.24 (1.07 to 1.44) (0.004)	0.88 (0.60 to 1.28) (0.495)	
Birth length (per cm increase)					0.96 (0.94 to 0.99) (0.004)	0.99 (0.92 to 1.06) (0.715)	
Apgar score at 5 min (per 1 point increase)					0.94 (0.84 to 1.053) (0.282)		
Multiple birth (vs single birth)	97.9 (8577/8760)	2.1 (183/8760)	7.7 (663/8577)	8.7 (16/183)	1.13 (0.69 to 1.86) (0.626)		
Caesarean delivery (vs vaginal delivery)	75.4 (6587/8731)	24.6 (2144/8731)	7.7 (506/6587)	8.1 (173/2144)	1.05 (0.88 to 1.25) (0.577)		
Non-Caucasian mother (vs Caucasian mother)	95.4 (8330/8734)	4.6 (404/8734)	7.7 (640/8330)	9.9 (40/404)	1.29 (0.94 to 1.77) (0.120)		
Non-Caucasian father (vs Caucasian father)	94.8 (8255/8708)	5.2 (453/8708)	7.5 (622/8255)	11.9 (54/453)	1.58 (1.20 to 2.09) (0.001)	1.44 (0.85 to 2.44) (0.180)	
Maternal age (per 1 year increase)					1.00 (0.98 to 1.01) (0.691)		
Paternal age, years (per 1 year increase)					1.00 (0.99 to 1.01) (0.918)		
Familial allergic disease							
Maternal self-reported, doctor-diagnosed allergic disease							
Any (yes vs no)	73.7 (6435/8732)	26.3 (2297/8732)	6.0 (386/6435)	12.6 (290/2297)	2.11 (1.81 to 2.45) (<0.001)	1.13 (0.84 to 1.52) (0.428)	
Asthma (yes vs no)	90.8 (809/8760)	9.2 (809/8760)	6.8 (544/7951)	16.7 (135/809)	2.44 (2.02 to 2.95) (<0.001)	1.30 (0.90 to 1.87) (0.158)	1.47 (1.12 to 1.93) (0.006)
Allergic rhinitis (yes vs no)	84.8 (7418/8749)	15.2 (1331/8749)	6.9 (511/7418)	12.5 (166/1331)	1.81 (1.52 to 2.16) (<0.001)		
Eczema (yes vs no)	88.1 (7706/8748)	11.9 (1042/8748)	7.0 (537/7706)	13.4 (140/1042)	1.93 (1.60 to 2.32) (<0.001)		
Paternal self-reported, doctor-diagnosed allergic disease							
Any (yes vs no)	78.7 (6611/8652)	21.3 (1841/8652)	6.3 (427/6811)	12.7 (234/1841)	2.03 (1.73 to 2.38) (<0.001)	1.32 (0.98 to 1.78) (0.067)	1.31 (1.04 to 1.65) (0.020)
Asthma (yes vs no)	92.9 (8083/8699)	7.1 (616/8699)	7.1 (571/8083)	15.4 (95/616)	2.18 (1.76 to 2.71) (<0.001)	0.73 (0.46 to 1.15) (0.174)	
Allergic rhinitis (yes vs no)	85.7 (7415/8657)	14.4 (1242/8657)	7.1 (528/7415)	11.2 (139/1242)	1.58 (1.30 to 1.90) (<0.001)		
Eczema (yes vs no)	93.8 (8153/8688)	6.2 (535/8688)	7.2 (587/8153)	14.8 (79/535)	2.05 (1.62 to 2.60) (<0.001)		

Continued

Table 4b Continued

	Unexposed % (n/N)	Exposed % (n/N)	Wheeze in unexposed % (n/N)	Wheeze in exposed % (n/N)	Unadjusted IRR (95% CI) (P value)	Primary model –adjusted IRR (95% CI) (P value) (n=3612)	Sensitivity model 1 – adjusted IRR (95% CI) (P value) (n=4227)
Living environment							
Rural housing (vs urban housing)	83.8 (7352/8774)	16.2 (1422/8774)	7.9 (580/7352)	7.1 (101/1422)	0.90 (0.73 to 1.11) (0.330)		
Mould in house (yes vs no)	90.2 (7667/8503)	9.8 (836/8503)	7.5 (573/7667)	9.5 (79836)	1.26 (1.00 to 1.60) (0.051)	0.96 (0.64 to 1.44) (0.833)	
Pets							
Any (yes vs no)	63.9 (5594/8751)	36.1 (3157/8751)	7.8 (4385594)	7.6 (241/3157)	0.98 (0.83 to 1.14) (0.752)		
Cat (yes vs no)	84.6 (7398/8751)	15.5 (1353/8751)	7.7 (566/7398)	8.4 (113/1353)	1.09 (0.89 to 1.34) (0.395)		
Dog (yes vs no)	83.9 (7343/8751)	16.1 (1408/8751)	8.3 (606/7343)	5.2 (73/1408)	0.63 (0.49 to 0.80) (<0.001)	0.90 (0.60 to 1.33) (0.587)	
Maternal education							
Basic not completed					1.16 (0.77 to 1.74) (0.483)		
Basic completed					Baseline comparator		
Junior college/vocational					1.15 (0.91 to 1.45) (0.255)		
College/university					1.20 (0.96 to 1.50) (0.114)		

Primary model: includes all variables with P<0.1 in univariate analysis (gender, gestation, birth weight, birth length, ethnicity of father, maternal allergy, maternal asthma, paternal allergy, paternal asthma, mould in house, dog ownership, day care attendance, respiratory tract infections and eczema), plus food allergy, variables related to feeding and smoke exposure and study centre.
Sensitivity model 1: This was generated by applying backward deletion to the primary model. It includes overlap of breast feeding/solids, mother smoking at 1-year follow-up, gender, day care attendance, LRTIs, eczema and study centre.
IRR, incidence rate ratio; LRTIs, lower respiratory tract infections; URTIs, upper respiratory tract infections.

Table 5 Association of significant risk factors from primary model with wheeze in the second year of life by centre

	Reykjavik	Southampton	Amsterdam	Berlin	Lodz	Vilnius	Madrid	Milan	Athens
n	829	494	569	1228	1084	1009	826		
Male gender (vs female)	1.45 (1.02 to 2.08) (0.040)	1.66 (1.00 to 2.76) (0.050)	3.17 (1.67 to 6.01) (<0.001)	1.72 (0.84 to 1.63) (0.346)	1.28 (0.45 to 3.65) (0.643)	2.00 (0.74 to 5.32) (0.167)	1.02 (0.46 to 2.25) (0.966)	–	–
Mother smoking at 1-year follow-up (yes vs no)	1.08 (0.64 to 1.83) (0.776)	2.72 (1.29 to 5.77) (0.009)	1.46 (0.75 to 2.86) (0.267)	1.27 (0.84 to 1.94) (0.258)	2.38 (0.75 to 7.55) (0.141)	0.79 (0.11 to 5.99) (0.823)	1.66 (0.68 to 4.05) (0.261)	–	–
Day care at any time in first 2 years of life (yes vs no)	1.30 (0.41 to 4.09) (0.656)	1.16 (0.69 to 1.95) (0.570)	1.71 (0.67 to 4.34) (0.258)	1.67 (1.01 to 2.77) (0.047)	1.91 (0.65 to 5.61) (0.237)	3.66 (1.47 to 9.13) (0.005)	0.69 (0.31 to 1.53) (0.365)	–	–
LRTIs in first year of life (≥quarterly vs ≤one)	1.83 (1.28 to 2.65) (0.001)	1.85 (0.82 to 4.17) (0.138)	2.33 (0.56 to 9.63) (0.243)	1.30 (0.63 to 2.66) (0.478)	2.48 (0.32 to 19.18) (0.384)	24.9 (2.98 to 207.18) (0.003)	2.42 (0.86 to 6.80) (0.093)	–	–
LRTIs in second year of life (≥quarterly vs ≤one)	2.74 (1.92 to 3.92) (<0.001)	1.07 (0.41 to 2.75) (0.896)	2.67 (0.65 to 11.15) (0.174)	1.29 (0.69 to 2.39) (0.421)	10.23 (1.34 to 78.33) (0.025)	–	11.83 (4.27 to 32.78) (<0.001)	–	–

Values represent: adjusted incidence rate ratio (95% CIs) (P value)*.
Where no figures are entered, Poisson estimation was not possible.
*Only significant associations from the primary model (gender, mother smoking at 1-year follow-up, day care attendance and LRTIs in the first and second years of life) were entered into the multivariable models for individual centres.
LRTIs, lower respiratory tract infections.

smoke exposure may help to reduce the burden of early childhood wheeze.

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Contributors ENCM was coordinator of the EuroPrevall project; KB was principal investigator of the birth cohort study. ACS, GR, KEG and AM performed the statistical analyses; TK, LG and VC provided statistical and epidemiological advice. ACS and GR drafted the manuscript. All authors reviewed and approved the final manuscript.

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Competing interests GR received grants from the EU FP6 Programme and UK Food Standards Agency during the conduct of the study; KEG has received educational grants from Nutrica and speaker fees from Nutrica and Abbott; TK has received grants from the EU FP7 Programme outside of the submitted work; STS received grants from Landspitali University Hospital Science Fund, GlaxoSmithKline and the Icelandic Student Innovation Fund during the conduct of the study and has received non-financial support from Novartis and Thermo Fisher outside of the submitted work; ENCM has received grants from the UK Biological and Biotechnological Sciences Research Council, DBV Technologies, Reacta Biotech Ltd, the Medical Research Council, Innovate and the North West Lung Centre Charity outside of the submitted work and is founding director of Reacta Biotech Ltd; KB has received funding for research activities from the European Union, German Research Foundation, Berliner Sparkasse, BEA-Stiftung, Food Allergy and Anaphylaxis Network, Food Allergy Initiative, Danone, Thermo Fisher and DST Diagnostics. Other authors have no competing interests to declare.

Ethics approval Ethics approval was obtained from the relevant ethics committee in each country involved in the study.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Brand PL, Baraldi E, Bisgaard H, *et al*. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008;32:1096–110.
2 Martinez FD, Wright AL, Taussig LM, *et al*. Asthma and Wheezing in the First Six Years of Life. *N Engl J Med Overseas Ed* 1995;332:133–8.
3 Ducharme FM, Tse SM, Chauhan B. Diagnosis, management, and prognosis of preschool wheeze. *Lancet* 2014;383:1593–604.
4 Asher MI, Weiland SK. The International Study of Asthma and Allergies in Childhood (ISAAC). *Clin Exp Allergy* 1998;28:52–66.
5 Janson C, Anto J, Burney P, *et al*. The European Community Respiratory Health Survey: what are the main results so far? *Eur Respir J* 2001;18:598–611.
6 Uphoff EP, Bird PK, Antó JM, *et al*. Variations in the prevalence of childhood asthma and wheeze in MeDALL cohorts in Europe. *ERJ Open Res* 2017;3:00150-2016–2016.
7 Arshad SH, Stevens M, Hide DW. The effect of genetic and environmental factors on the prevalence of allergic disorders at the age of two years. *Clin Exp Allergy* 1993;23:504–11.
8 Rancière F, Nikasinovic L, Bousquet J, *et al*. Onset and persistence of respiratory/allergic symptoms in preschoolers: new insights from the PARIS birth cohort. *Allergy* 2013;332:n/a–67.
9 Lodge CJ, Tan DJ, Lau MX, Mxz L, *et al*. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatr* 2015;104:38–53.
10 van Odijk J, Kull I, Borres MP, *et al*. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy* 2003;58:833–43.
11 Friedman NJ, Zeiger RS. The role of breast-feeding in the development of allergies and asthma. *J Allergy Clin Immunol* 2005;115:1238–48.
12 Oddy WH, Holt PG, Sly PD, *et al*. Association between breast feeding and asthma in 6 year old children: findings of a prospective birth cohort study. *BMJ* 1999;319:815–9.
13 Kull I, Wickman M, Lilja G, *et al*. Breast feeding and allergic diseases in infants-a prospective birth cohort study. *Arch Dis Child* 2002;87:478–81.
14 Zutavern A, von Mutius E, Harris J, *et al*. The introduction of solids in relation to asthma and eczema. *Arch Dis Child* 2004;89:303–8.
15 Snijders BE, Thijs C, van Ree R, *et al*. Age at first introduction of cow milk products and other food products in relation to infant atopic manifestations in the first 2 years of life: the KOALA Birth Cohort Study. *Pediatrics* 2008;122:e115–22.
16 Wang J, Liu AH. Food allergies and asthma. *Curr Opin Allergy Clin Immunol* 2011;11:249–54.
17 Keil T, McBride D, Grimshaw K, *et al*. The multinational birth cohort of EuroPrevall: background, aims and methods. *Allergy* 2010;65:482–90.
18 McBride D, Keil T, Grabenhenrich L, *et al*. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Pediatr Allergy Immunol* 2012;23:230–9.
19 Duijts L, Jaddoe VWV, van der Valk RJP, *et al*. Fetal exposure to maternal and paternal smoking and the risks of wheezing in preschool children: the Generation R Study. *Chest* 2012;141:876–85.
20 Pearce N, Sunyer J, Cheng S, *et al*. Comparison of asthma prevalence in the ISAAC and the ECRHS. *Eur Respir J* 2000;16:420–6.
21 Burney P, Chinn S, Jarvis D, *et al*. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survey (ECRHS). *Eur Respir J* 1996;9:687–95.
22 The International Study of Asthma and Allergies in Childhood (ISAAC) Steering Committee. Worldwide variations in the prevalence of asthma symptoms: the International Study of Asthma and Allergies in Childhood (ISAAC). *Eur Resp J* 1998;12:315–35.
23 Dharmage SC, Lowe AJ, Matheson MC, *et al*. Atopic dermatitis and the atopic march revisited. *Allergy* 2014;69:17–27.
24 Allen KJ, Dharmage SC. The role of food allergy in the atopic march. *Clin Exp Allergy* 2010;40:1439–41.
25 Malmberg LP, Saarinen KM, Pelkonen AS, *et al*. Cow's milk allergy as a predictor of bronchial hyperresponsiveness and airway inflammation at school age. *Clin Exp Allergy* 2010;40:1491–7.
26 Kurukulaaratchy RJ, Fenn MH, Waterhouse LM, *et al*. Characterization of wheezing phenotypes in the first 10 years of life. *Clin Exp Allergy* 2003;33:573–8.
27 McGowan EC, Bloomberg GR, Gergen PJ, *et al*. Influence of early-life exposures on food sensitization and food allergy in an inner-city birth cohort. *J Allergy Clin Immunol* 2015;135:171–8.
28 Caudri D, Wijga A, Scholtens S, *et al*. Early daycare is associated with an increase in airway symptoms in early childhood but is no protection against asthma or atopy at 8 years. *Am J Respir Crit Care Med* 2009;180:491–8.
29 Ball TM, Castro-Rodriguez JA, Griffith KA, *et al*. Siblings, day-care attendance, and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538–43.
30 Cheng G, Smith AM, Levin L, *et al*. Duration of day care attendance during infancy predicts asthma at the age of seven: the Cincinnati Childhood Allergy and Air Pollution Study. *Clin Exp Allergy* 2014;44:1274–81.
31 Nicolaou NC, Simpson A, Lowe LA, *et al*. Day-care attendance, position in sibship, and early childhood wheezing: a population-based birth cohort study. *J Allergy Clin Immunol* 2008;122:500–6.
32 Henderson AJ, Sherriff A, Northstone K, *et al*. Pre- and postnatal parental smoking and wheeze in infancy: cross cultural differences. *Eur Respir J* 2001;18:323–9.
33 Lux AL, Henderson AJ, Pocock SJ, *et al*. Wheeze associated with prenatal tobacco smoke exposure: a prospective, longitudinal study. ALSPAC Study Team. *Arch Dis Child* 2000;83:307–12.
34 Håberg SE, Stigum H, Nystad W, *et al*. Effects of pre- and postnatal exposure to parental smoking on early childhood respiratory health. *Am J Epidemiol* 2007;166:679–86.
35 Magnusson LL, Olesen AB, Wennborg H, *et al*. Wheezing, asthma, hayfever, and atopic eczema in childhood following exposure to tobacco smoke in fetal life. *Clin Exp Allergy* 2005;35:1550–6.
36 Grimshaw KE, Maskell J, Oliver EM, *et al*. Introduction of complementary foods and the relationship to food allergy. *Pediatrics* 2013;132:e1529–38.
37 Schoemaker AA, Sprikkelman AB, Grimshaw KE, *et al*. Incidence and natural history of challenge-proven cow's milk allergy in European children-EuroPrevall birth cohort. *Allergy* 2015;70:963–72.
38 Michel G, Silverman M, Strippoli MP, *et al*. Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J* 2006;28:1124–30.
39 Asher MI, Keil U, Anderson HR, *et al*. International Study of Asthma and Allergies in Childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483–91.

Appendix B

B.1 UBIOPRED Ethics Approval- Paediatric Study

South West London REC 1
Room 4W/12, 4th Floor West
Charing Cross Hospital
Fulham Palace Road
London
W6 8RF

Telephone: 020 331 17251
Facsimile: 020 331 17280

16 December 2010

Prof Andrew Bush
Professor of Paediatric Respiratory Medicine
Imperial College and Royal Brompton Hospital
Paediatric Respiratory Medicine
Sydney Street
London
SW3 6NP

Dear Prof Bush

Study Title: **U-BIOPRED: Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes. Assessment of children with severe asthma and severe pre-school Wheeze.**
REC reference number: **10/H0801/65**

The Research Ethics Committee reviewed the above application at the meeting held on 13 December 2010. Thank you for attending to discuss the study.

Ethical opinion

You confirmed that the research was part of a bigger study, and that vast amounts of data were being measured. It was hoped that correlations might define the subgroups of asthma, towards better treatment. Regarding the bronchoscopy, you explained that it would only be done if there was a definite clinical need. Clinical consent would be taken for the procedure, and this would include the usual tick boxes for samples to be used in research. Members learned that the tele-monitoring was being used in the pan-European study, but it was not clear whether or not this would be used at this site. It was agreed that keeping a diary could be burdensome, but you said it would be made as simple as possible, and participants did not need to use it if they found it to be too onerous. The Committee discussed the discomfort of the nasal spray, and you assured members that it would only be done once during the study and only if a child was anaesthised. It was understood that the control group would be moderate asthmatics, and that the multiple testing correct method had been used to work out the statistics. There would be a discovery cohort and a validation cohort. If the results delivered a 'handprint' of bio information then that would help future treatment. You agreed that the tissue bank in Southampton did not have an HTA licence, and assured members that the tissue would be stored by Imperial College, and that the correct material transfer paperwork would be completed if it went out of the hospital. The Committee asked that a storage of samples statement and transport be forwarded to them for the file, and you agreed to do this.

The members of the Committee present gave a **favourable ethical opinion** of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where the only involvement of the NHS organisation is as a Participant Identification Centre (PIC), management permission for research is not required but the R&D office should be notified of the study and agree to the organisation's involvement. Guidance on procedures for PICs is available in IRAS. Further advice should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Advertisement	1.0	17 July 2010
Participant Information Sheet: Teenagers - bronchoscopy	1.0	03 November 2010
Letter of invitation to participant	1	19 November 2010
GP/Consultant Information Sheets	1	19 November 2010
REC application	56713/167670/1/744	19 November 2010
Participant Consent Form: Paediatric Cohort Bronchoscopy information sheet for parents	1.0	03 November 2010

Participant Information Sheet: Teenagers - paediatric asthma cohort	1.0	03 November 2010
Participant Information Sheet: Paediatric Cohort, information sheet for parents	1.0	03 November 2010
Protocol	1 draft 15	09 October 2010
Covering Letter		25 November 2010
Letter from Sponsor	CRO1442	22 November 2010
IMI letter and Mandate	115010	
Investigator CV		09 August 2010
Participant Information Sheet: Paediatric Cohort Bronchoscopy information sheet for parents	1.0	03 November 2010
Participant Consent Form: Teenagers - paediatric asthma cohort	1.0	03 November 2010
Participant Consent Form: Teenagers - bronchoscopy	1.0	03 November 2010
Participant Consent Form: Paediatric Cohort, information sheet for parents	1.0	03 November 2010

Membership of the Committee
The members of the Ethics Committee who were present at the meeting are listed below.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/H0801/65	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Pp Dr Shelley Dolan
Chair

Email: Rosalind.cooke@imperial.nhs.uk

Enclosures: *"After ethical review – guidance for researchers*

Copy to: *Lucy Parker, Imperial College*

South West London REC 1
Attendance at Committee meeting on 13 December 2010

Committee Members:

Name	*Profession*	*Present*
Mr Roger Ahern	Medical Statistician	Yes
Dr. Sonya Babu-Narayan	Cardiologist Specialist Registrar	No
Mr Jeremy Butler	NHS Non Executive Director	Yes
Dr Robin Chung	Academic Medicine Trainee doctor and Engineer	Yes
Dr Shelley Dolan	Chief Nurse	Yes
Dr Adam Jacobs	Medical Statistician	No
Mr Simon Jordan	Consultant Thoracic Surgeon	No
Mr Philip Kimberley	Clinical Governance Information Manager	Yes
Mrs Patricia Pank	Retired University Lecturer	Yes
Dr Nazima Pathan	Consultant PICU	No
Mrs Paula Rogers	Research Nurse Manager	Yes
Ms Cate Savidge	CT Scanning Superintendent	No
Dr Elliot Shinebourne	Consultant Paediatric Cardiologist	Yes
Dr Mary Taj	Consultant Paediatric in Oncologist	Yes

Also in attendance:

Name	*Position (or reason for attending)*
Mrs Rosalind Cooke	Co-ordinator
Mrs Laura Royde	Observer

B.2 UBIOPRED Ethics Approval- Adult Study

NHS
National Research Ethics Service
East Central London REC 1
South House, Block A
Royal Free Hospital
Pond Street
London
NW3 2QG

Telephone: 020 7794 0552
Facsimile: 020 7794 0714

12 October 2010

Professor K F Chung
Professor of Respiratory Medicine
Imperial College London
National Heart and Lung Institute
Guy Scadding Building
Dovehouse Street
SW3 6LY

Dear Professor Chung

Study Title: **UBIOPRED: Unbiased BIOmarkers for the Prediction of REspiratory Disease Outcomes. Assessment of adults with severe asthma**
REC reference number: **10/H0721/66**

The Research Ethics Committee reviewed the above application at the meeting held on 15 September 2010. Thank you for attending to discuss the study.

Ethical opinion

The Committee reviewed the above study and the application was summarised by the lead reviewer.

You attended to discuss the application, were invited to join the meeting and was thanked for attending. A summary of the items discussed and the researchers' response to the issues raised is given below.

- The committee expressed to you that they were impressed with the study.
- The committee explained to you that they felt 33 pages for an information sheet was far too large and requested that it be broken down firstly in to participant categories, one for each (i.e. smokers, non-smokers and healthy volunteers) and secondly that the optional visits should also be on separate sheets. Professor Chung said he would do that.
- The committee explained to you that because the study was being sponsored commercially that the participants had a right to know and this should reflect in the information sheet.
- On the information sheet under the paragraph 'what rights do I have' the phrasing needs to be changed where it says' your study doctor.
- The committee had a long discussion with you regarding the informed consent template only being available in European languages which may possibly

exclude ethnic groups who may have a predisposition to the condition. You explained that there was no evidence that any one ethnic group had more chance of having Asthma than any other and that if the numbers were higher in one group there could be a possibility that they don't take medication. You explained that if this was the case then these particular participants were of no use to the study.

You then left the meeting.

Decision

The Committee gave the application a favourable opinion with the following additional conditions:

1. The information sheet needs to be broken down firstly in to participant categories, one for each (i.e. smokers, non-smokers and healthy volunteers) and secondly that the optional visits should also be on separate sheets. This documentation needs to be resub3mitted in the new format.

2. The fact that the study is being sponsored commercially should reflect in the information sheet.

3. On the information sheet under the paragraph 'what rights do I have' the phrasing needs to be changed where it says' your study doctor.

The REC nominated the Co-ordinator to be point of contact should further clarification be sought from the applicant upon receipt of the decision letter.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Investigator CV		16 September 2010
Protocol	1	01 August 2010
Advert severe Asthma	1	17 July 2010
Advert healthy	1	17 July 2010
REC application		24 August 2010
Covering Letter		19 August 2010
Letter from Sponsor		17 August 2010
Questionnaire	1	11 August 2010
Letter of invitation to participant	1	03 August 2010
GP/Consultant Information Sheets	1	09 August 2010
Participant Information Sheet	1	13 August 2010
Participant Consent Form	1	13 August 2010
Advert moderate Asthma		17 July 2010
Referees or other scientific critique report		17 August 2010

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

10/H0721/66	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr David Slovick
Chair

Email: john.doherty1@nhs.net

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers" [SL-AR1 for CTIMPs, SL-AR2 for other studies]

Copy to: *Ms Lucy Parker*
[R&D office for NHS care organisation at lead site]

East Central London REC 1

Attendance at Committee meeting on 15 September 2010

Committee Members:

Name	*Profession*	*Present*	*Notes*
Dr Waheeb Atia	Retired Consultant	Yes	
Ms Jill Bloom	Drug Information Pharmacist	Yes	
Dr Elizabeth Carrey	MSc Programme Director in Clinical Paediatrics	Yes	
Mrs Stephanie Cooper	Solicitor	Yes	
Mr Dan Ehrlich	Head of Optometry	No	
Mrs Ros Goldfarb	Retired Immigration Judge	No	
Mr Robert Goldstein	Economist	Yes	
Mr Hari Jayaram	Clinical Scientist in Ophthalmology	Yes	
Mr Peter Jones	Retired Head teacher	Yes	
Ms Sarah Kaiser	Director, Human Rights NGO	Yes	
Dr Stella Kingett	Consultant Psychiatrist	No	
Professor Diana Kornbrot	Professor of Mathematical Psychology	Yes	
Ms Mary Ryan	Personnel Manager	Yes	
Dr David Slovick	Consultant Physician	Yes	

B.3 Data Analysis Plan for Prospective Exacerbations in the UBIOPRED Study

Objectives

i. To assess whether baseline clinical clusters are associated with future asthma exacerbations.
ii. To assess whether baseline ISAC component atopy clusters are associated with future asthma exacerbations.
iii. To explore the characteristics of participants with more frequent severe asthma exacerbations. Characteristics may include demographic factors, previous asthma history, co-morbidities, allergic sensitisation, environmental exposures (e.g. allergens, irritants) and reported symptom triggers.

End Points

Exacerbation Rate:

The exacerbation rate (number of exacerbations per year) will be calculated from the number of exacerbations during the follow-up period divided by the number of months of follow-up multiplied by 12. This will be the primary outcome for the analysis.

The prospective exacerbation rate will be calculated separately for moderate, severe and life-threatening exacerbations to allow for sub-group analyses.

Definitions of Exacerbations:[112]

A moderate exacerbation will be defined as a deterioration in symptoms, lung function and/or an increase in bronchodilator use for at least 2 days, but not severe enough to require systemic corticosteroids or hospitalisation.

A severe asthma exacerbation will be defined as an asthma exacerbation involving at least one of the following:

- Use of systemic corticosteroids (oral or parenteral) or an increase from a stable maintenance dose for at least 3 days.
- An asthma-related hospitalisation or visit to the emergency department requiring oral corticosteroids (oral or parenteral, any duration).

For the purposes of this analysis, severe exacerbations will be divided into those requiring hospitalisation and those treated in an outpatient setting.

A life-threatening exacerbation will be defined as an intensive care unit (ICU) admission due to asthma requiring systemic corticosteroids (oral or parenteral, any duration).

Hypotheses

Null hypothesis 1: Future exacerbation rates do not differ between baseline clinical clusters of the UBIOPRED participants.

Alternative hypothesis 1: At least one of the baseline clinical clusters has a higher rate of future exacerbations.

Null hypothesis 2: Future exacerbation rates do not differ between ISAC component atopy clusters of the UBIOPRED participants.

Alternative hypothesis 2: At least one of the ISAC component atopy clusters has a higher rate of future exacerbations.

Participants

Only participants in the severe cohorts (adult A & B; paediatric A & C) will be included in this analysis since prospective exacerbation data is only available for these participants.

Data Required

Data required for this analysis:

- Questionnaire data collected at the baseline and longitudinal visits.
- Allergic sensitisation data – SPT and serum specific IgEs
- ISAC component atopy clusters
- Clinical clusters

Data will be downloaded from TRANSMART and will be quality checked before use in this analysis. No missing data will be imputed.

Baseline and Demographic Characteristics

For each adult and paediatric cohort, key baseline and demographic characteristics will be detailed. For example, age at assessment, gender, age at diagnosis, ethnicity and symptom triggers will be described. Continuous variables will be summarised using the mean and standard error or median and interquartile range and categorical variables will be summarised by counts and percentages. The characteristics of those with and without follow-up data will be compared.

Statistical Analysis

Primary Analyses: For each clinical cluster, exacerbation rates during the study follow-up period will be calculated. Histograms of exacerbation rates will be produced to determine the distribution of the data. If exacerbation rates are normally distributed, means and standard errors will be reported. If not, median and interquartile ranges will be reported. Differences between clusters will be assessed using analysis of variance (ANOVA) for normally distributed data or the Kruskal-Wallis test for non-parametric data. If a result is significant ($p < 0.05$), post hoc pairwise testing will be undertaken to determine which clusters are different from each other.

The same approach will be taken for the ISAC component atopy clusters.

Rates of exacerbations for each paediatric and adult cohort will also be calculated and compared in the same way. This will allow us to assess whether any of the clinical/ISAC atopy component clusters are more strongly associated with the future rate of exacerbations than the UBIOPRED cohort definitions.

Secondary Analyses: The association of specific clinical characteristics with future severe exacerbations (or all exacerbations if there are insufficient events) will be assessed using zero inflated Poisson regression or negative binomial regression. Factors which will be considered include demographic details, asthma history, co-morbidities, reported symptom triggers, allergic sensitisation and lung function. Factors with $p<0.1$ in univariate analysis will be entered into a backward multivariable model to identify independent risk factors for future severe exacerbations. Paediatric and adult participants will be analysed separately. Clinical clusters and ISAC component atopy clusters will be added to the multivariable models to determine whether they are associated with future severe exacerbations.

Software

Statistical analysis will be undertaken using Stata SE version 14.

B.4 UBIOPRED Paediatric Baseline Paper

ORIGINAL ARTICLE
ASTHMA AND PAEDIATRIC PULMONOLOGY

The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts

Louise Fleming[1,2,3], Clare Murray[4], Aruna T. Bansal[5], Simone Hashimoto[6], Hans Bisgaard[7,8], Andrew Bush[1,3,9,10], Urs Frey[11], Gunilla Hedlin[12], Florian Singer[13,14], Wim M. van Aalderen[15], Nadja H. Vissing[7,8], Zaraquiza Zolkipli[16,17,18], Anna Selby[16,17,18], Stephen Fowler[4,19], Dominick Shaw[20], Kian Fan Chung[1,2,3], Ana R. Sousa[21], Scott Wagers[22], Julie Corfield[23,24], Ioannis Pandis[25], Anthony Rowe[26], Elena Formaggio[27], Peter J. Sterk[6] and Graham Roberts[16,17,18] on behalf of the U-BIOPRED Study Group[28]

Affiliations: [1]National Heart and Lung Institute, Imperial College London, London, UK. [2]NIHR Biomedical Research Unit, Royal Brompton and Harefield NHS Trust, London, UK. [3]NIHR Biomedical Research Unit, Royal Brompton NHS Trust, London, UK. [4]Centre for Respiratory Medicine and Allergy, The University of Manchester, Manchester Academic Health Science Centre, University Hospital of South Manchester NHS Foundation Trust, Manchester, UK. [5]Acclarogen Ltd, St John's Innovation Centre, Cambridge, UK. [6]Dept of Respiratory Medicine, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. [7]Copenhagen Prospective Studies on Asthma in Childhood, Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. [8]Gentofte Hospital, University of Copenhagen, Copenhagen, Denmark. [9]Dept of Paediatrics, Imperial College London, London, UK. [10]Dept of Respiratory Paediatrics, Royal Brompton Hospital, London, UK. [11]University Children's Hospital Basel UKBB, University of Basel, Basel, Switzerland. [12]Dept of Women's and Children's Health and Center for Allergy Research, Karolinska Institutet at Karolinska University Hospital, Stockholm, Sweden. [13]University Children's Hospital Zurich, Zurich, Switzerland. [14]University Children's Hospital Bern, Bern, Switzerland. [15]Dept of Paediatric Respiratory Medicine and Allergy, Emma Children's Hospital, Academic Medical Centre, University of Amsterdam, Amsterdam, The Netherlands. [16]NIHR Southampton Respiratory Biomedical Research Unit, Clinical and Experimental Sciences and Human Development and Health, Southampton, UK. [17]Faculty of Medicine, University of Southampton, Southampton, UK. [18]The David Hide Asthma and Allergy Research Centre, St Mary's Hospital, Isle of Wight, UK. [19]Airways Clinic, Lancashire Teaching Hospitals NHS Foundation Trust, Preston, UK. [20]Respiratory Research Unit, University of Nottingham, Nottingham, UK. [21]Respiratory Therapeutic Unit, GlaxoSmithKline, Stockley Park, UK. [22]BioSci Consulting, Maasmechelen, Belgium. [23]AstraZeneca R&D, Mölndal, Sweden. [24]Areteva, Nottingham, UK. [25]Data Science Institute, South Kensington Campus, Imperial College London, London, UK. [26]Janssen R&D Ltd, High Wycombe, UK. [27]CROMSOURCE, Verona, Italy. [28]For a full list of members of the U-BIOPRED Study Group, please refer to the Acknowledgements section.

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@ERSpublications
Children with severe preschool wheeze or severe asthma are usually atopic and have impaired quality of life http://ow.ly/RrrGE

This article has supplementary material available from erj.ersjournals.com

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This study is registered on ClinicalTrials.gov (NCT01982162).

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Conflict of interest: Disclosures can be found alongside the online version of this article at erj.ersjournals.com

Appendix B

ABSTRACT U-BIOPRED aims to characterise paediatric and adult severe asthma using conventional and innovative systems biology approaches.

A total of 99 school-age children with severe asthma and 81 preschoolers with severe wheeze were compared with 49 school-age children with mild/moderate asthma and 53 preschoolers with mild/moderate wheeze in a cross-sectional study.

Despite high-dose treatment, the severe cohorts had more severe exacerbations compared with the mild/moderate ones (annual medians: school-aged 3.0 *versus* 1.1, preschool 3.9 *versus* 1.8; p<0.001). Exhaled tobacco exposure was common in the severe wheeze cohort. Almost all participants in each cohort were atopic and had a normal body mass index. Asthma-related quality of life, as assessed by the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ), was worse in the severe cohorts (mean±SE school-age PAQLQ: 4.77±0.15 *versus* 5.80±0.19; preschool PACQLQ: 4.27±0.18 *versus* 6.04±0.18; both p≤0.001); however, mild/moderate cohorts also had significant morbidity. Impaired quality of life was associated with poor control and airway obstruction. Otherwise, the severe and mild/moderate cohorts were clinically very similar.

Children with severe preschool wheeze or severe asthma are usually atopic and have impaired quality of life that is associated with poor control and airflow limitation: a very different phenotype from adult severe asthma. In-depth phenotyping of these children, integrating clinical data with high-dimensional biomarkers, may help to improve and tailor their clinical management.

Introduction

Asthma is one of the most common chronic diseases in childhood. Although many achieve control with currently available therapies, an estimated 5–10% of patients remain symptomatic despite receiving large amounts of treatment. These children with severe asthma [1] have poor quality of life (QoL), frequent asthma attacks and lung function impairment, are at high risk of side-effects from medication and account for significant medical and societal costs.

It is increasingly recognised that asthma, and particularly severe asthma, is not one single disease entity. Data in adults have been available for some time [2] but evidence now exists in children to suggest that there are a number of different clinical manifestations of severe asthma that are driven by a variety of pathophysiological mechanisms [3, 4]. Phenotypic classifications in children have primarily been based on clinical data, lung function measurement and assessment of allergic status. The small number of studies that have included biological samples have described important differences in the underlying pathobiology of severe asthma in children compared with adults [5–7]. Some but not all preschool children with severe wheeze have evidence of airway remodelling and inflammation from an early age [8], consistent with established asthma, but little is known about the underlying mechanisms, which in many cases appear to be very different from school-age and adult asthma. These early changes do not always predict a progression to asthma [9]. These observations are indicative of considerable heterogeneity amongst children with severe school-aged asthma or severe preschool wheeze.

In order to capture the relevant phenotypes of children with severe asthma or severe wheeze, careful and extensive clinical characterisation is required. This provides the basis for future integration with biological disease markers. The Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) project is a public–private partnership, within the framework of the Innovative Medicines Initiative, bringing together academic institutions and European Federation of Pharmaceutical Industries and Associations partners from across Europe. It was set up in 2009 in order to take advantage of the combination of extensive clinical characterisation and biological fingerprinting by "omics" technologies for the unbiased discovery of phenotypes in both adult and paediatric severe asthma [10]. The paediatric arm of the U-BIOPRED study used the same thorough clinical characterisation and innovative systems biology approach as the adult study [11] to integrate clinical, physiological and inflammatory data and patient/parent-reported outcomes with the high-dimensional data of "omics" technologies (transcriptomic, proteomic, lipidomic and metabolomic) obtained from blood, urine, breath and airway samples [12].

The main objective of this first report of the paediatric U-BIOPRED study was to fully clinically characterise the cohorts of children with severe asthma and preschool wheeze and mild/moderate cohorts based on cross-sectional baseline data. The second objective was to investigate the burden of severe asthma and severe preschool wheeze, as described by QoL, and the clinical factors that relate to this burden.

Methods

This was a multicentre, prospective, observational cohort study following the life course of asthma. Full details of the adult cohorts are described in the companion paper by Shaw *et al.* [11].

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Cohorts

Seven centres in five European countries recruited preschool (age 1–5 years) and school-age (age 6–17 years) children. Four paediatric cohorts were recruited by approaching consecutive patients attending respiratory and general paediatric clinics who fulfilled the following inclusion criteria. 1) Severe school-aged asthma (SA): ongoing poorly controlled asthma (persistent symptoms, frequent exacerbations or persistent airflow limitation) despite high-dose inhaled corticosteroids (ICS) and at least two other controller medications [13]. 2) Mild/moderate school-aged asthma (MMA): controlled or partly controlled asthma, prescribed low-dose ICS and no other or one additional controller medication. 3) Severe preschool wheeze (SW): persistent symptoms and frequent exacerbations despite current or failed high-dose ICS and a leukotriene receptor antagonist (LTRA). 4) Mild/moderate preschool wheeze (MMW): controlled or partially controlled symptoms prescribed no treatment or low-dose ICS and/or a LTRA.

Full cohort descriptions, inclusion and exclusion criteria are shown in table S2 in the online supplementary material. All children in the severe cohorts (SA and SW) had been under follow-up with a respiratory paediatrician for ⩾6 months before enrolling in the study. During this time, assessments were undertaken to exclude other diagnoses, treat comorbidities, optimise asthma control, assess medication adherence (*e.g.* checking prescription uptake) and reduce allergen exposure in sensitised individuals [13].

Study design

The study was approved by the local ethics committees (see table S1). Parents/caregivers provided written consent; children gave assent where appropriate. The study is registered with ClinicalTrials.gov (NCT01982162). The study aims and outcomes have been published on the U-BIOPRED website (www.europeanlung.org/en/projects-and-research/projects/u-biopred/home).

All participants were identified and recruited locally and attended a screening and baseline visit. Clinical data and biological samples were collected from all cohorts (figure 1). Full details are provided in the supplementary material.

Study assessments

Baseline data included demographics, current and past medical history (including detailed asthma and atopic disease history), medications, birth history, family history, exposure to environmental tobacco smoke, and known clinical and environmental risk factors. Asthma control was assessed using the Asthma Control Test (ACT) (for children ⩾12 years of age) [14] or the Childhood Asthma Control Test (cACT) for children <12 years [15]. Non-scheduled healthcare utilisation was assessed by documenting exacerbations. QoL was assessed using the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) (school-aged children only) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire

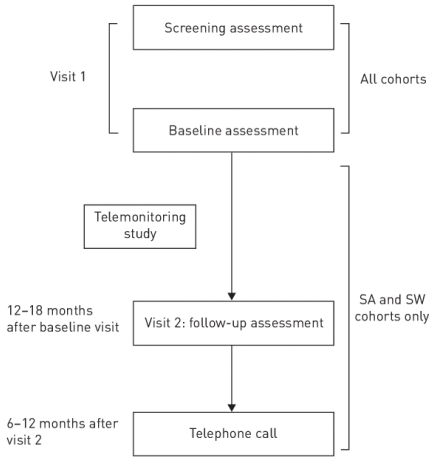

FIGURE 1 Visit schedule. SA: severe school-aged asthma; SW: severe preschool wheeze.

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(PACQLQ) [16, 17]. Adherence was evaluated using the Medication Adherence Report Scale (MARS) [18]. A summary of the assessments carried out in each of the cohorts is shown in table S3.

In all cohorts, total IgE, specific IgE tests and/or skin prick testing (SPT) to six common allergens were undertaken. Atopy was defined as the presence of sensitisation on SPT (wheal ⩾3 mm) or serum specific IgE (⩾0.35 $kU \cdot L^{-1}$). Spirometry before and after bronchodilator [19] and exhaled nitric oxide fraction (F_{ENO}) were measured where possible. Sputum induction was performed in the school-aged cohorts and differential cell counts were determined. Exposure to environmental tobacco smoke was assessed by measuring urinary cotinine. In selected centres, forced oscillation technique and plethysmography were undertaken.

Full details of the methods are provided in the supplementary material, including samples collected for future "omic" analysis. A centralised biobank was selected for the study and operated in accordance with its own Standard Operating Procedures, as described in the supplementary material.

Data management and statistics

Data were entered into an electronic clinical record form before being transferred to the tranSMART system for quality control checks [20]. Missing data were not imputed.

The cohort sizes of 97 and 43 (comparing SA and MMA), and 77 and 54 (comparing SW and MMW), both provide 80% power to detect a difference in means of half a standard deviation, assuming standard normally distributed data, in a two-sided t-test at the 5% significance level [21].

Due to the descriptive (as opposed to inferential) nature of the analyses presented, raw, unadjusted p-values are reported throughout the manuscript. Those in tables 1–4 were derived using logistic regression (binary variables) or general linear regression (continuous variables). Continuous variables exhibiting positive skew were summarised by the median and interquartile range (IQR), and were log-transformed prior to association testing. Where appropriate, tests of association were performed both with and without adjustments for age and sex.

Associations between key potential facets of asthma (forced expiratory volume in 1 s (FEV_1) z-score, FEV_1/forced vital capacity (FVC), age of onset/diagnosis, number of exacerbations in preceding 12 months, ACT z-score, body mass index (BMI), MARS, hay fever, eczema, atopy, smoking and white race) were each assessed singly for association with asthma burden, as quantified by QoL, using linear regression. Adjustments for age and sex were not applied at this stage due to a lack of univariate association between either age or sex. QoL contour plots were derived for continuous variables with $p<0.05$, using two-dimensional kernel density estimation with a bivariate normal kernel, evaluated at 50 grid points in each direction [23]. The variables were also modelled jointly in a multivariate general linear model. Backwards stepwise regression using the Akaike Information Criterion was then applied, in order to derive a parsimonious model.

Analyses were performed using R version 2.15.2 (R Core Team; www.r-project.org). The present report is based on cross-sectional analysis of the baseline data.

Results

Participants

A total of 298 children and teenagers with asthma or wheeze were screened to recruit 282 participants. Numbers of participants in each cohort that provided baseline questionnaire data, spirometry, blood samples and sputum samples are detailed in figure 2.

Cohorts SA and MMA were well matched for age (mean 12.21 and 11.26 years, respectively), as were cohorts SW and MMW (mean 3.56 and 3.46 years, respectively). Exposure to environmental tobacco smoke was reported by 15.8–22.8% of each cohort. More of the SW cohort were positive for urinary cotinine than of the MMW cohort (19.4% *versus* 4.3%; p=0.035) (table 1).

Atopy

Most of the school-age participants in both cohorts (SA, MMA) were atopic (85.4% and 89.5%, respectively) (table 1). Rates of atopy were lower in both preschool cohorts (36.5% and 37.5%) (table 1). The majority of the school-age children (SA, MMA) had a diagnosis of eczema, hay fever or allergic rhinitis (table 1). Most of the preschoolers had a co-existing diagnosis of eczema with a third also having allergic rhinitis. In the preschool children, significantly more SW than MMW participants had a diagnosis of hay fever (58.8% *versus* 36.1%, respectively; p=0.04). A sizeable minority of participants reported symptoms of food allergy (40.2% for SA *versus* 32.6% for MMA, p=0.39; 21.1% for SW *versus* 27.8% for MMW, p=0.38).

TABLE 1 Baseline demographic characteristics and medical history

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Demographic details						
Female	46/97 (47.4%)	16/43 (37.2%)	0.263	27/77 (35.1%)	20/54 (37.0%)	0.817
Age years	12.21±0.31 (n=97)	11.26±0.48 (n=43)	0.583[f]	3.56±0.14 (n=77)	3.46±0.16 (n=54)	0.410[f]
White	74/97 (76.3%)	32/43 (74.4%)	0.812	62/77 (80.5%)	48/54 (88.9%)	0.204
Mother smoked during pregnancy	14/94 (14.9%)	6/43 (14.0%)	0.885	10/77 (13.0%)	1/54 (1.9%)	0.052
Smoker	0/97 (0.0%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Second-hand smoke exposure	21/92 (22.8%)	9/43 (20.9%)	0.805	12/76 (15.8%)	9/54 (16.7%)	0.893
Urinary cotinine present	8/93 (8.6%)	5/38 (13.2%)	0.432	12/62 (19.4%)	2/46 (4.3%)	0.035
Anthropometry[#]						
Height cm	152.82±1.65 (n=97)	148.12±2.58 (n=43)	0.604[f]	102.88±1.13 (n=76)	103.62±1.52 (n=53)	0.068[f]
Height z-score	0.68±0.34 (n=97)	0.58±0.2 (n=43)	0.851	1.14±0.16 (n=76)	1.53±0.18 (n=53)	0.108
Weight kg	51.74±1.85 (n=97)	43.64±2.3 (n=43)	0.067[f]	17.63±0.48 (n=77)	17.27±0.46 (n=53)	0.687[f]
Weight z-score	1.14±0.21 (n=97)	0.66±0.19 (n=43)	0.167	0.94±0.14 (n=77)	0.92±0.13 (n=53)	0.930
BMI kg·m^{-2}	21.52±0.5 (n=97)	19.21±0.5 (n=43)	0.035[f]	16.56±0.25 (n=76)	15.99±0.15 (n=53)	0.071[f]
BMI z-score	0.99±0.13 (n=97)	0.56±0.17 (n=43)	0.058	0.26±0.15 (n=76)	−0.04±0.1 (n=53)	0.133
Past medical history						
Mode of delivery						
Normal vaginal	68/97 (70.1%)	28/43 (65.1%)	0.558	59/77 (76.6%)	38/54 (70.4%)	0.422
Instrumental	5/97 (5.2%)	3/43 (7.0%)	0.669	4/77 (5.2%)	3/54 (5.6%)	0.928
Caesarian	24/97 (24.7%)	12/43 (27.9%)	0.693	14/77 (18.2%)	13/54 (24.1%)	0.413
Breast feeding months	5.09±0.8 (n=97)	5.72±1.13 (n=43)	0.659	4.61±0.62 (n=77)	7.43±0.81 (n=54)	0.006
Admitted to neonatal unit	14/97 (14.4%)	3/43 (7.0%)	0.223	7/77 (9.1%)	3/54 (5.6%)	0.457
Other medical problems						
Diagnosed hay fever	75/91 (82.4%)	33/40 (82.5%)	0.991	30/51 (58.8%)	13/36 (36.1%)	0.039
Diagnosed eczema	77/95 (81.1%)	28/40 (70.0%)	0.162	42/57 (73.7%)	32/40 (80.0%)	0.473
Diagnosed allergic rhinitis	61/93 (65.6%)	29/38 (76.3%)	0.232	22/52 (42.3%)	11/36 (30.6%)	0.265
Diagnosed gastro-oesophageal reflux[¶]	19/94 (20.2%)	3/40 (7.5%)	0.081	8/58 (13.8%)	11/40 (27.5%)	0.097
Diagnosed vocal cord dysfunction	2/94 (2.1%)	1/40 (2.5%)	0.894	0/59 (0.0%)	0/40 (0.0%)	NA
Reported food allergy[+]	39/97 (40.2%)	14/43 (32.6%)	0.390	16/76 (21.1%)	15/54 (27.8%)	0.376
Allergic sensitisation						
Positive skin prick test	69/83 (83.1%)	33/37 (89.2%)	0.395	22/65 (33.8%)	18/48 (37.5%)	0.688
Positive specific IgE	40/47 (85.1%)	21/24 (87.5%)	0.784	14/30 (46.7%)	13/26 (50.0%)	0.803
Atopy[§]	70/82 (85.4%)	34/38 (89.5%)	0.540	23/63 (36.5%)	18/48 (37.5%)	0.915

Data are presented as n/N (%) or mean±SE, unless otherwise stated. p-values were calculated using Pearson's Chi-squared test or a Kruskall–Wallis test. BMI: body mass index; NA: not applicable. [#]: anthropometry z-scores generated using the 1990 British growth data [22]; [¶]: gastro-oesophageal reflux was diagnosed on the basis of suggestive symptoms, pH monitoring, endoscopy or response to therapy; [+]: symptoms of reported food allergy represent symptoms of urticaria, angioedema, pruiritis, throat tightness, stridor, chest tightness or wheeze within 2 h of contact with food; [§]: atopy defined as a positive skin prick test (≥3 mm) or a positive specific IgE (≥0.35 kU·L^{-1}); [f]: p-values adjusted for age and sex.

Asthma history and treatment

The mean age at diagnosis was in the fourth year of life for both school-aged cohorts, whereas for the preschool ones it was in their second year (table 2). There were significant differences in the triggers for respiratory symptoms between the severe and mild/moderate cohorts (table 2). While almost all of cohorts SA, MMA and SW were treated with ICS, they were prescribed for less than half of MMW as most had failed to respond to ICS therapy. Additionally, 23.7% of SA and 5.2% of SW were receiving maintenance oral corticosteroid therapy. Parent/participant-reported adherence to therapy was good in all cohorts (table 2).

Lung function and airway inflammation

Lung function and bronchodilator reversibility in the SA and MMA cohorts were similar at baseline when participants were well (table 3). For preschool participants able to perform spirometry, results were again similar for severe and mild/moderate cohorts. There was a trend towards specific airway conductance being lower in the SA cohort compared with the MMA cohort (1.58 *versus* 1.95 kPa·s^{-1}; p=0.054) (table 3). We were only able to collect induced sputum from a minority of school-aged participants so we could not make a meaningful comparison between the cohorts (table 3).

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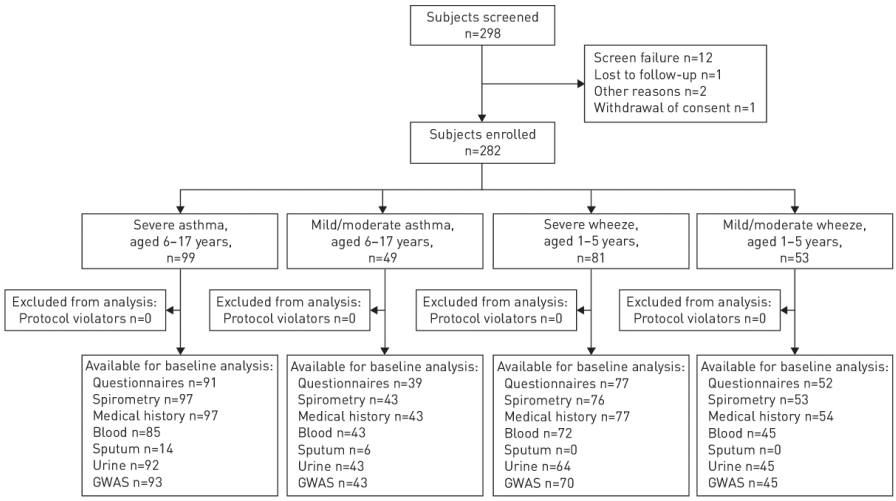

FIGURE 2 Consort diagram for participants in the paediatric Unbiased Biomarkers for the Prediction of Respiratory Disease Outcomes (U-BIOPRED) study. GWAS: genome-wide association study.

Asthma burden: QoL, control and exacerbations

Asthma-related QoL was used as the primary measure of burden. The mean result for the PAQLQ for the SA cohort was 4.77, equivalent to "somewhat bothered", significantly worse than for the MMA cohort (5.8, equivalent to "bothered a bit"; p<0.001). Similar differences were found for the symptoms, emotions and activity domains (table 4). For the preschool cohorts the PACQLQ was used as a proxy, given that there is no validated QoL tool for preschool wheeze. For SW the mean was 4.27 ("some of the time" or "somewhat worried/concerned"), again significantly worse than for MMW (6.04, "hardly ever" or "hardly worried/concerned"; p<0.001).

The burden of asthma was also illustrated by the ACT results, which assessed ongoing symptoms and rescue medication. Most of the severe cohorts were uncontrolled (74.6% in SA compared with 29.2% in MMA, p<0.001; 78.0% in SW compared with 18.2% in MMW, p<0.001). This was reflected in the number of exacerbations in the year prior to assessment. In the previous year, the SA cohort had a median of three exacerbations (IQR two to five), compared with one (IQR zero to two) in the MMA cohort (p<0.001). A similar difference was seen between the SW and MMW cohorts (table 4). However, there was still an important asthma burden in the mild/moderate cohorts.

Which factors are associated with asthma burden?

Asthma burden is described as asthma-related QoL, with z-scores used to give a combined variable for all age groups. Pre-bronchodilator FEV_1, but not FEV_1/FVC ratio, was significantly related to QoL (regression coefficient 0.151, p=0.002) (table 5). The number of exacerbations in the previous year was significantly inversely associated with asthma QoL (−0.52, p<0.001). Asthma control (measured by ACT and cACT z-score) was significantly related to asthma QoL (0.730, p<0.001). BMI was inversely associated with asthma QoL (−0.036, p=0.011). These are illustrated in figure 3. Results were similar when PAQLQ and PACQLQ were considered separately (table S4).

To assess which factors were independent predictors of asthma-related QoL, a backward stepwise regression analysis was performed for FEV_1 z-score, FEV_1/FVC, age of onset/diagnosis, number of exacerbations in preceding 12 months, ACT z-score, BMI, MARS, hay fever, eczema, atopy, smoking and white race. Significant factors in the reduced model were ACT z-score (regression coefficient 0.76, p<0.001) and FEV_1 z-score (0.11, p=0.036).

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TABLE 2 Asthma history and treatment

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Basis of asthma definition						
Airway hyperresponsiveness, PC_{20} <8 mg·mL^{-1}	0/97 (0%)	6/43 (14%)	NA	NA	NA	NA
Bronchodilator reversibility, FEV_1 ⩾12%	66/97 (68%)	19/43 (44.2%)	0.028	NA	NA	NA
Persistent airflow limitation, FEV_1 z-score <−1.96	5/96 (5.2%)	NA	NA	NA	NA	NA
Spontaneous variability, FEV_1 ⩾12%	1/97 (1%)	19/43 (44.2%)	0.994	NA	NA	NA
Diurnal peak flow variability, ⩾15%	8/97 (8.2%)	2/43 (4.7%)	0.452	NA	NA	NA
Basis of severe asthma definition						
High-dose ICS and trials of other controllers	97/97 (100%)	NA	1.000	NA	NA	NA
Persistent symptoms	63/96 (65.6%)	NA	NA	NA	NA	NA
Frequent exacerbations	44/96 (45.8%)	NA	NA	NA	NA	NA
Persistent airflow limitation	5/96 (5.2%)	NA	NA	NA	NA	NA
Maintenance oral corticosteroids	19/96 (19.8%)	NA	NA	NA	NA	NA
Symptom control						
Controlled	NA	22/43 (51.2%)	NA	NA	37/52 (71.2%)	NA
Partially controlled	NA	21/43 (48.8%)	NA	NA	15/52 (28.8%)	NA
Asthma history						
Age at diagnosis years	3.25±0.27 (n=93)	3.78±0.48 (n=41)	0.305	1.74±0.12 (n=73)	1.48±0.13 (n=46)	0.146
ICU admission ever	19/97 (19.6%)	4/43 (9.3%)	0.139	9/77 (11.7%)	2/54 (3.7%)	0.124
ICU admission in past year	5/97 (5.2%)	1/43 (2.3%)	0.458	6/77 (7.8%)	2/54 (3.7%)	0.347
Intubation ever	12/96 (12.5%)	1/43 (2.3%)	0.090	5/77 (6.5%)	2/54 (3.7%)	0.490
Reported triggers for respiratory symptoms						
Respiratory infections	91/96 (94.8%)	41/42 (97.6%)	0.465	77/77 (100.0%)	53/53 (100.0%)	1.000
Pets	62/92 (67.4%)	29/38 (76.3%)	0.315	14/60 (23.3%)	11/49 (22.4%)	0.913
Routine physical activities	44/94 (46.8%)	8/42 (19.0%)	0.003	29/77 (37.7%)	5/54 (9.3%)	<0.001
Physical exercise	86/96 (89.6%)	33/42 (78.6%)	0.090	58/74 (78.4%)	20/51 (39.2%)	<0.001
Aspirin	3/53 (5.7%)	1/22 (4.5%)	0.845	1/51 (2.0%)	0/41 (0.0%)	NA
Cold air	79/97 (81.4%)	24/42 (57.1%)	0.003	61/72 (84.7%)	24/53 (45.3%)	<0.001
Pollutants	55/85 (64.7%)	17/37 (45.9%)	0.055	18/55 (32.7%)	5/47 (10.6%)	0.011
Perfumes	42/90 (46.7%)	23/41 (56.1%)	0.318	20/67 (29.9%)	3/51 (5.9%)	0.003
Wood smoke	41/78 (52.6%)	19/39 (48.7%)	0.695	16/55 (29.1%)	5/46 (10.9%)	0.030
Dust	75/93 (80.6%)	28/42 (66.7%)	0.080	35/70 (50.0%)	13/50 (26%)	0.009
Barns	39/71 (54.9%)	13/28 (46.4%)	0.446	15/50 (30.0%)	4/47 (8.5%)	0.012
Stress	55/92 (59.8%)	18/43 (41.9%)	0.053	24/63 (38.1%)	5/51 (9.8%)	0.001
Menstrual cycle	7/87 (8.0%)	3/40 (7.5%)	0.916	0/72 (0.0%)	0/48 (0.0%)	NA
Pollen	76/93 (81.7%)	31/42 (73.8%)	0.296	34/65 (52.3%)	9/49 (18.4%)	<0.001
Fungus	37/72 (51.4%)	15/34 (44.1%)	0.485	17/53 (32.1%)	6/46 (13.0%)	0.030
Early viral wheeze	0/97 (0.0%)	0/43 (0.0%)	NA	5/77 (6.5%)	18/54 (33.3%)	<0.001
Multi-trigger wheeze	94/97 (96.9%)	43/43 (100.0%)	0.995	72/77 (93.5%)	35/54 (64.8%)	<0.001
Asthma therapy						
Short-acting β-agonist	95/97 (97.9%)	42/43 (97.7%)	0.921	75/77 (97.4%)	40/54 (74.1%)	<0.001
Nebulised β-agonist	22/97 (22.7%)	2/43 (4.7%)	0.019	16/77 (20.8%)	2/54 (3.7%)	0.013
ICS						
Any dose	97/97 (100.0%)	43/43 (100.0%)	1.000	75/77 (97.4%)	24/54 (44.4%)	<0.001
⩽400 µg BUD equivalent	0/96 (0.0%)	42/43 (97.7%)	NA	0/69 (0.0%)	24/26 (92.3%)	NA
⩾800 µg BUD equivalent	96/96 (100.0%)	0/43 (0.0%)	NA	69/69 (100.0%)	2/26 (7.7%)	NA
Oral corticosteroids	23/97 (23.7%)	0/43 (0.0%)	NA	4/77 (5.2%)	0/54 (0.0%)	NA
Any LABA	94/97 (96.9%)	25/43 (58.1%)	<0.001	34/77 (44.2%)	0/54 (0.0%)	NA
Inhaled combination LABA/ICS	89/97 (91.8%)	25/43 (58.1%)	<0.001	32/77 (41.6%)	0/54 (0.0%)	NA
Anti-cholinergic	14/97 (14.4%)	2/43 (4.7%)	0.112	7/77 (9.1%)	3/54 (5.6%)	0.457
Leukotriene modifier	71/97 (73.2%)	10/43 (23.3%)	<0.001	58/77 (75.3%)	20/54 (37%)	<0.001
Xanthine	15/97 (15.5%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Cromones	10/97 (10.3%)	1/43 (2.3%)	0.140	1/77 (1.3%)	0/54 (0.0%)	NA
Antibiotic therapy	17/97 (17.5%)	1/43 (2.3%)	0.036	10/77 (13.0%)	5/54 (9.3%)	0.511
Mucolytic	2/97 (2.1%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
Anti-IgE therapy	17/97 (17.5%)	0/43 (0.0%)	NA	0/77 (0.0%)	0/54 (0.0%)	NA
SMART regime	23/97 (23.7%)	4/43 (9.3%)	0.055	0/77 (0.0%)	0/54 (0.0%)	NA
Adherence to therapy, MARS total	22.76±0.23 (n=94)	21.3±0.48 (n=43)	0.003	22.85±0.26 (n=73)	22.18±0.43 (n=44)	0.161

Data are presented as n/N (%) or mean±SE, unless otherwise stated. p-values were calculated using a two-sample t-test or a Chi-squared test. PC_{20}: provocative concentration causing a 20% fall in FEV_1; FEV_1: forced expiratory volume in 1 s; ICS: inhaled corticosteroids; ICU: intensive care unit; BUD: budesonide; LABA: long-acting β-agonist; SMART: Symbicort Maintenance and Reliever Therapy (AstraZeneca); MARS: Medication Adherence Report Scale (a five-item self-report scale for assessment of adherent behaviour including unintentional and intentional non-adherence; each item was answered using a five-graded response scale (very often (1) to never (5)), so low scores indicate low levels of adherence); NA: not applicable.

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TABLE 3 Lung function and airway inflammation

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Lung function						
FEV_1 pre-salbutamol						
% predicted	88.68±2.15 (n=96)	93.51±2.47 (n=42)	0.186	104.34±3.21 (n=19)	99.23±5.29 (n=10)	0.390
z-score	−0.92±0.18 (n=96)	−0.53±0.2 (n=42)	0.190	0.33±0.24 (n=19)	−0.03±0.4 (n=10)	0.421
FVC pre-salbutamol						
% predicted	102.15±1.65 (n=96)	104.45±2.02 (n=42)	0.418	107.99±3.5 (n=19)	103.54±5.23 (n=10)	0.473
z-score	0.16±0.14 (n=96)	0.37±0.17 (n=42)	0.381	0.55±0.25 (n=19)	0.25±0.38 (n=10)	0.487
FEV_1/FVC ratio	0.77±0.01 (n=97)	0.8±0.02 (n=42)	0.169	0.91±0.02 (n=19)	0.89±0.02 (n=10)	0.678
Absolute % change in FEV_1 with salbutamol	12.36±1.41 (n=84)	8.98±1.44 (n=42)	0.133	7.89±2.33 (n=15)	9.43±3.39 (n=6)	0.724
Total lung capacity L	4.69±0.12 (n=97)	4.28±0.19 (n=43)	0.073	1.05±0.08 (n=76)	1.09±0.11 (n=53)	0.748
Specific airway conductance $kPa \cdot s^{-1}$	1.58±0.1 (n=54)	1.95±0.18 (n=23)	0.054	2.03±NA (n=1)	3.16±NA (n=1)	NA
Airway inflammation						
Exhaled nitric oxide ppb	33.5 (15.4–62.2) (n=92)	35.84 (14–41) (n=38)	0.152	NA	NA	NA
Sputum eosinophils absolute	5.5 (2.2–14) (n=14)	16.5 (2.8–17.2) (n=4)	0.944	NA	NA	NA
Sputum eosinophils %	1.06 (0.4–2.7) (n=14)	3.34 (0.5–3.5) (n=4)	0.927	NA	NA	NA
Sputum neutrophils absolute	151.5 (77.8–354.5) (n=14)	224.25 (187–306.2) (n=4)	0.645	NA	NA	NA
Sputum neutrophils %	32.55 (16.6–68.7) (n=14)	43.11 (34.5–61) (n=4)	0.670	NA	NA	NA

Data are presented as mean±SE or median (interquartile range), unless otherwise stated. Predictive lung function equations from QUANJER *et al.* [19] were used to generate predicted values and z-scores. p-values were calculated using a Kruskall–Wallis test. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; NA: not applicable.

TABLE 4 Asthma quality of life, exacerbations and control

	School-aged			Preschool		
	Severe asthma cohort	Mild/moderate asthma cohort	p-value	Severe wheeze cohort	Mild/moderate wheeze cohort	p-value
Patients n	97	43		77	54	
Asthma-related quality of life						
PAQLQ						
Total mean	4.77±0.15 (n=91)	5.8±0.19 (n=39)	<0.001	NA	NA	NA
Total z-score	−0.22±0.1 (n=91)	0.51±0.14 (n=39)	<0.001	NA	NA	NA
Symptoms	4.57±0.16 (n=91)	5.77±0.19 (n=39)	<0.001	NA	NA	NA
Emotion	4.91±0.18 (n=91)	6.03±0.19 (n=39)	<0.001	NA	NA	NA
Activity limitation	3.91±0.15 (n=91)	4.57±0.19 (n=39)	0.012	NA	NA	NA
PACQLQ						
Total	NA	NA	NA	4.27±0.18 (n=77)	6.04±0.18 (n=52)	<0.001
Total z-score	NA	NA	NA	−0.46±0.09 (n=77)	0.66±0.12 (n=52)	<0.001
Exacerbations						
Exacerbations in previous year	3 (2–5) (n=97)	1.05 (0–2) (n=43)	<0.001	3.91 (1–6) (n=77)	1.83 (0–2.8) (n=54)	<0.001
Asthma control						
ACT >12 years						
Total	15.49±0.63 (n=67)	20.25±0.81 (n=24)	<0.001	NA	NA	NA
Total z-score	−0.25±0.12 (n=67)	0.69±0.17 (n=24)	<0.001	NA	NA	NA
Total ⩽19	50/67 (74.6%)	7/24 (29.2%)	<0.001	NA	NA	NA
Childhood ACT						
Total	16.38±0.98 (n=29)	19.22±1.01 (n=18)	0.061	15.2±0.79 (n=41)	23±0.67 (n=22)	<0.001
Total z-score	−0.26±0.16 (n=29)	0.23±0.2 (n=18)	0.065	−0.47±0.13 (n=41)	1.01±0.16 (n=22)	<0.001
Total ⩽19	19/29 (65.5%)	7/18 (38.9%)	0.078	32/41 (78%)	4/22 (18.2%)	<0.001
Combined ACT[#]						
z-score	−0.26±0.1 (n=95)	0.47±0.13 (n=41)	<0.001	−0.47±0.13 (n=41)	1.01±0.16 (n=22)	<0.001

Data are presented as mean±SE, median (interquartile range) or n/N (%), unless otherwise stated. p-values were calculated using a Kruskall–Wallis test or Pearson's Chi-squared test. PAQLQ: Paediatric Asthma Quality of Life Questionnaire; PACQLQ: Paediatric Asthma Caregiver's Quality of Life Questionnaire; ACT: Asthma Control Test (used for participants >12 years; children aged 4–11 years completed the childhood ACT); NA: not applicable. [#]: to allow the joint analysis of the ACT and childhood ACT, data were transformed to improve symmetry and then z-scores were calculated.

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TABLE 5 Factors associated with asthma burden as measured by quality of life

	Sample size n	Regression coefficient	95% confidence interval	p-value
FEV_1 z-score#	161	0.151	0.05–0.25	0.002
FEV_1/FVC	162	1.113	−0.23–2.46	0.104
Age of onset years	247	0.028	−0.03–0.08	0.311
Specific airway conductance $kPa \cdot s^{-1}$	78	0.132	−0.16–0.42	0.366
Log exacerbations in previous year	263	−0.523	−0.67–−0.38	<0.001
ACT combined z-score	196	0.730	0.63–0.83	<0.001
BMI $kg \cdot m^{-2}$	261	−0.036	−0.06–−0.01	0.011
MARS total	249	−0.040	−0.09–0.01	0.097
Hay fever diagnosed	211	−0.281	−0.57–0.01	0.057
Eczema diagnosed	225	−0.116	−0.42–0.19	0.452
Atopy	225	−0.070	−0.34–0.2	0.612
Second-hand smoke	257	−0.022	−0.33–0.29	0.890
White ethnicity	263	0.346	0.04–0.66	0.028

Data represent linear regression analyses looking at the association between each factor and quality of life. Quality of life was assessed by the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) or the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ). To allow the joint analysis of the PAQLQ and PACQLQ, data were transformed to improve symmetry and then z-scores were calculated. In order to make maximum use of the data, plethysmography (specific airway conductance), with 194 missing values, was excluded from joint modelling. Analysis was also performed for PAQLQ and PACQLQ separately (results in the online supplementary material). FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; ACT: Asthma Control Test; BMI: body mass index; MARS: Medication Adherence Report Scale. #: predictive lung function equations from QUANJER *et al.* [19] were used to generate z-scores.

Discussion

This article presents the detailed clinical characteristics of 282 children in four paediatric cohorts, including preschool and school-age children with both severe and mild/moderate wheeze and asthma across Europe. Standard Operating Procedures and Good Clinical Practice criteria were used to ensure consistency and quality across sites, with data collected on an online platform and stored in a single online repository. The severe cohorts by definition had a significantly higher treatment burden than the mild/moderate ones, despite which they remained poorly controlled with frequent severe exacerbations and low ACT scores. Children with severe disease, and their caregivers, had significantly lower QoL scores across all domains than the mild/moderate cohorts. Asthma control and airway obstruction were found to be significantly associated with QoL. Exposure to environmental tobacco smoke in the SW cohort was a striking finding and will be an important concomitant factor in future analyses. Otherwise, the severe and mild/moderate cohorts were very similar; this is in contrast to the adult severe and mild/moderate U-BIOPRED cohorts [11] and suggests that paediatricians should be cautious about extrapolating from adult studies. The vast majority of children were atopic. The rates of reported food allergy were high, although the rate of actual food allergy is expected to be much lower [24]. Most had a normal BMI, unlike the typical adult severe asthma phenotype. Also conspicuous was the morbidity in the mild/moderate paediatric groups; although they were clearly differentiated from the severe groups, a number are clearly sub-optimally treated. These data demonstrate that we succeeded in recruiting severe paediatric cohorts and provide a comprehensive view of the clinical burden of severe asthma or wheeze in childhood.

Children in the U-BIOPRED SA and SW cohorts have frequent symptoms and severe exacerbations that adversely impact on QoL and carry a high treatment burden; almost 17% of the SA cohort was prescribed omalizumab and 24% prescribed maintenance oral corticosteroids. This is in keeping with a previous study, which reported a strong association between health-related QoL and ACT score in children with problematic severe asthma [25]. In our study, the impact on QoL was seen to be greatest for the SW cohort. A significant impact on QoL was also seen in the mild/moderate cohorts, highlighting the often overlooked influence of asthma on the lives of children and their families. Allergic sensitisation and other atopic diseases were a frequent finding across all cohorts, more so in school-aged children, adding further to the treatment burden. Lung function was not significantly different in the school-aged cohorts, possibly due to good treatment adherence, being between exacerbations and the fact that FEV_1 is not a good discriminator of severity.

U-BIOPRED builds upon previous severe asthma cohort studies [26–29]. However, to our knowledge, this is the first study to recruit preschool wheeze cohorts on the basis of a consensus definition, which can be

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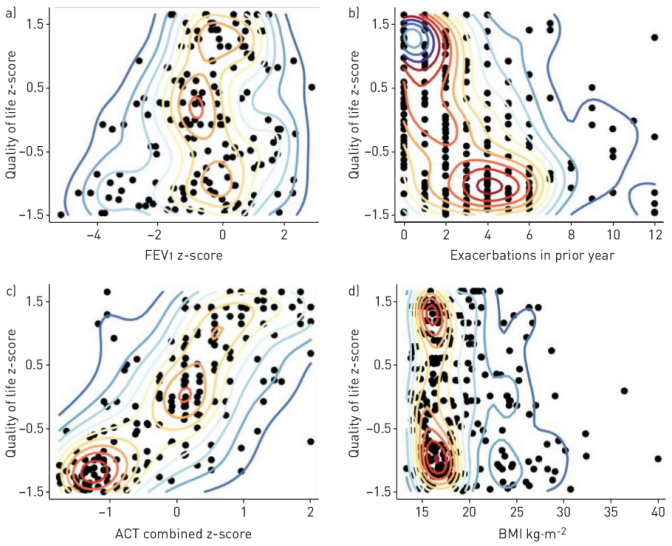

FIGURE 3 Factors associated with asthma burden as measured by quality of life. Figures represent scatter plots describing the relationship between each factor and the combined asthma-related quality of life z-score. The contour lines are coloured blue to red, to indicate increasing density of points in the graph to overcome the issue of overlying data points. The contour plots show c) a strong positive relationship between quality of life and asthma control (Asthma Control Test (ACT)) with a) a weaker positive relationship between quality of life and lung function (forced expiratory volume in 1 s (FEV_1) z-score). Additionally there is b) a strong negative relationship between quality of life and exacerbation rate plus d) a weaker negative relationship between quality of life and body mass index (BMI). The combined z-score merges the Paediatric Asthma Quality of Life Questionnaire (PAQLQ) and the Paediatric Asthma Caregiver's Quality of Life Questionnaire (PACQLQ). The density was modelled using two-dimensional kernel density estimation.

directly compared with parallel school-age and adult cohorts [13]. Most studies of preschool wheeze have been based on birth cohorts. A small number of studies have focused on severe preschool wheeze [9, 30] and they have provided valuable insights into the underlying pathophysiology and natural history of preschool wheeze. In common with the TENOR (The Epidemiology and Natural History of Asthma: Outcomes and Treatment Regimens) [27] and SARP (Severe Asthma Research Program) [26, 28] severe asthma cohorts, U-BIOPRED children with severe asthma were commonly atopic, had high healthcare utilisation and a high treatment burden. In the TENOR study there were far more boys than girls (63% *versus* 37%) in the severe cohort but, in common with SARP, we did not see these sex differences. Unlike SARP, children in the SA cohort did not have significantly higher F_{ENO} levels than those in the MMA cohort; however, F_{ENO} measurements were made off-line in SARP, making it difficult to make direct comparisons.

There are a number of limitations to this study. There were no healthy controls recruited to the paediatric cohorts; however, as the aim was to understand what makes asthma severe, the mild/moderate cohorts are the most appropriate comparator. The mild/moderate asthma group were all on prophylactic medication and participants were recruited from general paediatric and respiratory clinics so they are not completely representative of the children with mild/moderate asthma or wheeze seen in primary care. Also, as this is a multicentre pan-European study, it is likely that there were differences in patients recruited into each cohort between centres. Feasibility and safety considerations meant that assessments such as airway hyperresponsiveness were not included. Additionally, preschool children were unable to perform lung function, induced sputum and F_{ENO}. We were not able to reach the target of 100 preschool severe wheeze children; many had not been under tertiary follow-up for ⩾6 months, did not reach the treatment threshold or did not meet the stringent inclusion criteria at screening due to the intermittent nature of

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their symptoms. There was no objective measure of adherence during the study; however, this was a pragmatic study of real-life severe asthma where clinics had tried to exclude adherence issues, and the high MARS scores suggest a good level of adherence.

Despite advances in recent years in our understanding and management of severe asthma, the data presented here highlight the ongoing unmet needs. Both severe asthma and severe wheeze are heterogeneous diseases. Single or even clustered biomarkers have had limited impact in predicting clinical course or therapeutic efficacy in children: for example, the SA cohort is not distinguishable from MMA by classical lung function and airway inflammatory phenotypes [5, 7, 9]. Classification of preschool wheeze phenotypes is at an even more basic level, limited to symptom pattern [31, 32] and progression to asthma determined retrospectively. Analysis of samples from these cohorts will provide high-dimensional biological ("omics") data, which can be integrated with clinical characteristics to define multidimensional handprints of severe asthma. This approach has the potential to allow a step change in our understanding of asthma, identify more relevant prognostic and therapeutic targets and enable a personalised, phenotype-driven approach to management to address the unmet burden.

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References

1 ChungKF, Wenzel SE, Brozek JL, *et al.* International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014; 43: 343–373.
2 Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006; 368: 804–813.
3 Fitzpatrick AM, Teague WG, Meyers DA, *et al.* Heterogeneity of severe asthma in childhood: confirmation by cluster analysis of children in the National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011; 127: 382–389.
4 Just J, Gouvis-Echraghi R, Rouve S, *et al.* Two novel, severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J* 2012; 40: 55–60.
5 Bossley CJ, Fleming L, Gupta A, *et al.* Pediatric severe asthma is characterized by eosinophilia and remodeling without $T_{H}2$ cytokines. *J Allergy Clin Immunol* 2012; 129: 974–982.
6 Hastie AT, Moore WC, Meyers DA, *et al.* Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010; 125: 1028–1036.
7 Fleming L, Tsartsali L, Wilson N, *et al.* Sputum inflammatory phenotypes are not stable in children with asthma. *Thorax* 2012; 67: 675–681.
8 Saglani S, Payne DN, Zhu J, *et al.* Early detection of airway wall remodeling and eosinophilic inflammation in preschool wheezers. *Am J Respir Crit Care Med* 2007; 176: 858–864.
9 O'Reilly R, Ullmann N, Irving S, *et al.* Increased airway smooth muscle in preschool wheezers who have asthma at school age. *J Allergy Clin Immunol* 2013; 131: 1024–1032.
10 Auffray C, Adcock IM, Chung KF, *et al.* An integrative systems biology approach to understanding pulmonary diseases. *Chest* 2010; 137: 1410–1416.
11 Shaw DE, Sousa AR, Fowler SJ, *et al.* Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J* 2015; 46: 1308–1321.

 DOI: 10.1183/13993003.00780-2015

Appendix B

12 Wheelock CE, Goss VM, Balgoma D, *et al.* Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur Respir J* 2013; 42: 802–825.
13 Bel EH, Sousa A, Fleming L, *et al.* Diagnosis and definition of severe refractory asthma: an international consensus statement from the Innovative Medicine Initiative (IMI). *Thorax* 2011; 66: 910–917.
14 Nathan RA, Sorkness CA, Kosinski M, *et al.* Development of the Asthma Control Test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004; 113: 59–65.
15 Liu AH, Zeiger R, Sorkness C, *et al.* Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol* 2007; 119: 817–825.
16 Juniper EF, Guyatt GH, Feeny DH, *et al.* Measuring quality of life in children with asthma. *Qual Life Res* 1996; 5: 35–46.
17 Juniper EF, Guyatt GH, Feeny DH, *et al.* Measuring quality of life in the parents of children with asthma. *Qual Life Res* 1996; 5: 27–34.
18 Cohen JL, Mann DM, Wisnivesky JP, *et al.* Assessing the validity of self-reported medication adherence among inner-city asthmatic adults: the Medication Adherence Report Scale for Asthma. *Ann Allergy Asthma Immunol* 2009; 103: 325–331.
19 Quanjer PH, Stanojevic S, Cole TJ, *et al.* Multi-ethnic reference values for spirometry for the 3–95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012; 40: 1324–1343.
20 Szalma S, Koka V, Khasanova T, *et al.* Effective knowledge management in translational medicine. *J Transl Med* 2010; 8: 68.
21 Cohen J. Statistical Power Analysis for the Behavioral Sciences. 2nd Edn. Hillsdale, Lawrence Erlbaum Associates, 1988.
22 1990 British growth data. Available from www.healthforallchildren.com/?product=lmsgrowth Date last accessed: April 26, 2015.
23 Venables WN, Ripley BD. Modern Applied Statistics with S. 4th Edn. New York, Springer, 2002.
24 Nwaru BI, Hickstein L, Panesar SS, *et al.* The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy* 2014; 69: 62–75.
25 Nordlund B, Konradsen JR, Pedroletti C, *et al.* The clinical benefit of evaluating health-related quality-of-life in children with problematic severe asthma. *Acta Paediatr* 2011; 100: 1454–1460.
26 Fitzpatrick AM, Gaston BM, Erzurum SC, *et al.* Features of severe asthma in school-age children: atopy and increased exhaled nitric oxide. *J Allergy Clin Immunol* 2006; 118: 1218–1225.
27 Chipps BE, Szefler SJ, Simons FE, *et al.* Demographic and clinical characteristics of children and adolescents with severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2007; 119: 1156–1163.
28 Moore WC, Bleecker ER, Curran-Everett D, *et al.* Characterization of the severe asthma phenotype by the National Heart, Lung, and Blood Institute's Severe Asthma Research Program. *J Allergy Clin Immunol* 2007; 119: 405–413.
29 Konradsen JR, Nordlund B, Lidegran M, *et al.* Problematic severe asthma: a proposed approach to identifying children who are severely resistant to therapy. *Pediatr Allergy Immunol* 2011; 22: 9–18.
30 Saglani S, Nicholson AG, Scallan M, *et al.* Investigation of young children with severe recurrent wheeze: any clinical benefit? *Eur Respir J* 2006; 27: 29–35.
31 Brand PL, Baraldi E, Bisgaard H, *et al.* Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008; 32: 1096–1110.
32 Brand PL, Caudri D, Eber E, *et al.* Classification and pharmacological treatment of preschool wheezing: changes since 2008. *Eur Respir J* 2014; 43: 1172–1177.

DOI: 10.1183/13993003.00780-2015

References

1. Bush A, Grigg J, Saglani S. Managing wheeze in preschool children. *BMJ* 2014;348:348-54.

2. Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. Asthma and wheezing in the first six years of life. *N Engl J Med* 1995;332(3):133-38.

3. Masoli M, Fabian D, Holt S, Beasley R. The global burden of asthma: executive summary of the GINA Dissemination Committee Report. *Allergy* 2004;59(5):469-78.

4. Papadopoulos NG, Arakawa H, Carlsen KH, Custovic A, Gern J, Lemanske RF, et al. International consensus on (ICON) pediatric asthma *Allergy* 2012;67:976-97.

5. Olin JT, Wechsler ME. Asthma: pathogenesis and novel drugs for treatment. *BMJ* 2014;349.

6. Triggiani M, Jutel M, Knol EF. The Underlying Mechanisms of Asthma, In: Global Atlas of Asthma In: Akdis CA, Agache I (eds.) *Global Atlas of Asthma* Zurich: European Academy of Allergy and Clinical Immunology 2013 p31-33.

7. Holgate ST, Arshad SH, Roberts G, Howarth PH, Thurner P, Davies DE. A new look at the pathogenesis of asthma. *Clinical Science* 2010;118:439-50.

8. Wahn U. Natural History of Asthma, In: Global Atlas of Asthma In: Akdis CA, Agache I (eds.) *Global Atlas of Asthma* Zurich: European Academy of Allergy and Clinical Immunology 2013 p21-22.

9. Ducharme FM, Tse SM, Chauhan B. Diagnosis, management and prognosis of preschool wheeze. *Lancet* 2014;383:1593-604.

10. Brand PLP, Baraldi E, Bisgaard H, Boner AL, Castro-Rodriguez JA, Custovic A. ERS Task Force. Definition, assessment and treatment of wheezing disorders in preschool children: an evidence-based approach. *Eur Respir J* 2008;32:1096-110.

11. Gibeon D, Chung KF. The investigation of severe asthma to define phenotypes. *Clin Exp Allergy* 2012;42:678-92.

12. Jackson DJ, Sykes A, Mallia P, Johnston SL. Asthma exacerbations: Origin, effect and prevention. *J Allergy Clin Immunol* 2011;128(1165-74):1165-74.

13. Savenije OE, Granell R, Caudri D, Koppelman GH, Smit HA, Wijga A, et al. Comparison of childhood wheezing phenotypes in 2 birth cohorts: ALSPAC and PIAMA. *J Allergy Clin Immunol* 2011;127:1505-12.

14. Brand PLP, Caudri D, Eber E, Gaillard EA, Garcia-Marcos L, Hedlin G, et al. Classification and pharmacological treatment of preschool wheezing: changes since 2008. *Eur Respir J* 2014;43:1172-77.

15. Kurukulaaratchy RJ, Fenn MH, Waterhouse LM, Matthews SM, Holgate ST, Arshad SH. Characterization of wheezing phenotypes in the first 10 years of life. *Clin Exp Allergy* 2003;33:573-78.

16. Collins SA, Pike KC, Inskip HM, Godfrey KM, Roberts G, Holloway JW, et al. Validation of novel wheeze phenotypes using longitudinal airway function and atopic sensitization data in the first 6 years of life: evidence from the Southampton Women's survey. *Pediatric Pulmonology* 2013;48(7):683-92.

17. Castro-Rodriguez JA, Holberg CJ, Wright AL, Martinez FD. A Clinical Index to Define Risk of Asthma in Young Children with Recurrent Wheezing *Am J Respir Crit Care Med* 2000;162:1403-06.

18. Castro-Rodriguez JA. The Asthma Predictive Index: A very useful tool for predicting asthma in young children. *J Allergy Clin Immunol* 2010;126:212-6.

19. Guilbert TW, Morgan WJ, Zeiger RS, Mauger DT, Boehmer SJ, Szefler SJ, et al. Long-Term Inhaled Corticosteroids in Preschool Children at High Risk for Asthma *N Engl J Med* 2006;354(19):1985-97.

20. Murray CS, Woodcock A, Langley SJ, Morris J, Custovic A, IFWIN study team. Secondary prevention of asthma by use of inhaled fluticasone diproprionate in wheezy infants (IWWIN): double-blind, randomised controlled study. *Lancet* 2006;368:754-62.

21. Martinez FD. Inhaled corticosteroids and asthma prevention *Lancet* 2006;368:708-10.

22. Asher MI, Weiland SK. The International Study of Asthma and Allergies in Childhood (ISAAC). *Clin Exp Allergy* 1998;28(Suppl 5):52-66.

23. European Community Respiratory Health Survey. Variations in the prevalence of respiratory symptoms, self-reported asthma attacks, and use of asthma medication in the European Community Respiratory Health Survery (ECRHS). *Eur Respir J* 1996;9:687-95.

24. Burney PGJ, Luczynska C, Chinn S, Jarvis D. The European Community Respiratory Health Survey. *Eur Respir J* 1994;7:954-60.

25. Burr ML, Limb ES, Andrae S, Barry DM, Nagel F. Childhood Asthma in Four Countries: A Comparative Survery. *Int J Epidemiol* 1994;23:341-7.

26. Leung R, Ho P. Asthma, allergy and atopy in three south-east Asian populations. *Thorax* 1994;49:1205-10.

27. Braback L, Breborowicz A, Dreborg S, Knutsoson A, Pieklik H, Bjorksten B. Atopic sensitisation and respiratory symptoms among Polish and Swedish school children. *Clin Exp Allergy* 1994;24:826-35.

28. Pearce N, Sunyer J, Cheng S, Chinn S, Bjorksten B, Burr M, et al. Comparison of asthma prevalence in the ISAAC and ECRHS. *Eur Respir J* 2000;16:420-26.

29. Uphoff EP, Bird PK, Anto JM, Basterrechea M, Von Berg A, Bergstrom A, et al. Variations in the prevalence of childhood asthma and wheeze in MeDALL cohorts in Europe. *Eur J Open Res* 2017;3:00150-2016.

30. Henderson AJ, Sherriff A, Northstone K, Kukla L, ALSPAC Study Team, ELSPAC Co-ordinating Centre. Pre- and postnatal parental smoking and wheeze in infancy: cross cultural differences *Eur Respir J* 2001;18:323-29.

31. Bousquet J, Gern JE, Martinez FD, Anto JM, Johnson CC, Holt PG, et al. Birth cohorts in asthma and allergic diseases: report of a NIAID/NHLBI/MeDALL joint workshop. *J Allergy Clin Immunol* 2014;133(6):1535-46.

32. Papadopoulos NG, Kalobatsou A. Respiratory viruses in childhood asthma. *Current Opinion in Allergy & Clinical Immunology* 2007;7:91-95.

33. Lemanske RF, Busse WW. Asthma: Factors underlying inception, exacerbation and disease progression. *J Allergy Clin Immunol* 2006;117:S456-61.

34. Kusel MMH, de Klerk NH, Holt PG, Kebadze T, Johnston SL, Sly PD. Role of Respiratory Viruses in Acute Upper and Lower Respiratory Tract Illness in the First Year of Life: A Birth Cohort Study. *Pediatr Infect Dis J* 2006;25:680-86.

35. Wolf DG, Greenberg D, Kalkstein D, Shemer-Avni Y, Givon-Lavi N, Saleh N, et al. Comparison of Human Metapneumovirus, Respiratory Syncytial Virus and Influenza A Lower Respiratory Tract Infections in Hospitalized Young Children *Pediatr Infect Dis J* 2006;25:320-24.

36. Lemanske RF, Jackson DJ, Gangnon RE, Evans MD, Li Z, Shult PA, et al. Rhinovirus illnesses during infancy predict subsequent childhood wheezing *J Allergy Clin Immunol* 2005;116:571-7.

37. Sigurs N, Bjarnaosn R, Sigurbergsson F, Kjellman B. Respiratory syncytial virus bronchiolitis in infancy is an important risk factor for asthma and allergy at age 7. *Am J Respir Crit Care Med* 2000;161:1501-7.

38. Caudri D, Wijga A, Scholtens S, Kerkhof M, Gerritsen J, Ruskamp JM, et al. Early Daycare is Associated with an Increase in Airway Symptoms in Early Childhood but Is No Protection against Asthma or Atopy at 8 Years. *Am J Respir Crit Care Med* 2009;180:491-98.

39. Ball TM, Castro-Rodriguez JA, Griffith KA, Holberg CJ, Martinez FD, Wright AL. Siblings, day-care attendance and the risk of asthma and wheezing during childhood. *N Engl J Med* 2000;343:538-43.

40. Ranciere F, Nikasinovic L, Bousquet J, Momas I. Onset and persistence of allergic or respiratory symptoms in preschoolers: new insights from the PARIS birth cohort *Allergy* 2013;68:1158-67.

41. Nicolaou NC, Simpson A, Lowe LA, Murray CS, Woodcock A, Custovic A. Day-care attendance, position in sibship, and early childhood wheezing: A population-based birth cohort study. *J Allergy Clin Immunol* 2008;122:500-6.

42. Strachan DP, Cook DG. Parental smoking and lower respiratory illness in infancy and early childhood. *Thorax* 1997;52:905-14.

43. Strachan DP, Cook DG. Parental smoking and childhood asthma: longitudinal and case-control studies *Thorax* 1998;53:204-12.

44. Neuman A, Hohmann C, Orsini N, Pershagen G, Eller E, Kjaer HF, et al. Maternal Smoking in Pregnancy and Asthma in Preschool Children: A Pooled Analysis of Eight Birth Cohorts. *Am J Respir Crit Care Med* 2012;186(10):1037-43.

45. Duijts L, Jaddoe VWV, van der Valk RJP, Henderson JA, Hofman A, Raat H, et al. Fetal Exposure to Maternal and Paternal Smoking and the Risks of Wheezing in Preschool Children. *Chest* 2012;141(4):876-85.

46. Lux AL, Henderson AJ, Pocock SJ, Team AS. Wheeze associated with prenatal tobacco smoke exposure: a prospective, longitudinal study. *Arch Dis Child* 2000;83(4):307-12.

47. Haberg SE, Stigum H, Nystad W, Nafstad P. Effects of Pre- and Postnatal Exposure to Parental Smoking on Early Childhood Respiratory Health *Am J Epidemiol* 2007;166(6):679-86.

48. Landau LI. Parental smoking: asthma and wheezing illnesses in infants and children. *Paediatric Respiratory Reviews* 2001;2:202-06.

49. Friedman N, Zeiger RS. The role of breast-feeding in the development of allergies and asthma *J Allergy Clin Immunol* 2005;115:1238-48.

50. van Odijk J, Kull I, Borres MP, Brandtzaeg P, Edberg U, Hanson LA, et al. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1996-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations *Allergy* 2003;58:833-43.

51. Kull I, Wickman M, Lilja G, Nordvall SL, Pershagen G. Breastfeeding and allergic diseases in infants- a prospective birth cohort study. *Arch Dis Child* 2002;87:478-81.

52. Kull I, Almqvist C, Lilja G, Pershagen G, Wickman M. Breast-feeding reduces the risk of asthma during the first 4 years of life. *J Allergy Clin Immunol* 2004;114:755-60.

53. Oddy WH, Peat JK, De Klerk NH. Maternal asthma, infant feeding and the risk of asthma in childhood *J Allergy Clin Immunol* 2002;110:65-7.

54. Forsyth JS, Ogston SA, Clark AF, C, Howie PW. Relation between early introduction of solid food to infants and their weight and illnesses during the first two years of life. *BMJ* 1993;306:1572-6.

55. Wilson AC, Forsyth JS, Greene SA, Irvine L, Hau C, Howie PW. Relation of infant diet to childhood health: seven year follow up of cohort of children in Dundee infant feeding study. *BMJ* 1998;316:21-5.

56. Zutavern A, von Mutius E, Harris J, Mills P, Moffatt S, White C, et al. The introduction of solids in relation to asthma and eczema. *Arch Dis Child* 2004;89:303-08.

57. Snijders BEP, Thijs C, van Ree R, van den Brandt PA. Age at First Introduction of Cow Milk Products and Other Food Products in Relation to Infant Atopic Manifestatons in the First 2 Years of Life: The KOALA Birth Cohort Study *Pediatrics* 2008;122(1):115-22.

58. Johansson SGO, Bieber T, Dahl R, Friedman P, Lanier BQ, Lockey RF, et al. Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J Allergy Clin Immunol* 2004;113:832-6.

59. Custovic A, Lazic N, Simpson A. Pediatric asthma and development of atopy. *Current Opinion in Allergy & Clinical Immunology* 2013;13:173-80.

60. Sly PD, Boner AL, Bjorksten B, Bush A, Eigenmann P, Gern JE, et al. Early identification of atopy in prediction of persistent asthma in children. *Lancet* 2008;372:1100-06.

61. Arshad SH, Kurukulaaratchy RJ, Fenn M, Matthews S. Early Life Risk Factors for Current Wheeze, Asthma and Bronchial Hyperresponsiveness *Chest* 2005;127(2):502-08.

62. Illi S, Von Mutius E, Lau S, Niggemann B, Gruber C, Wahn U. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet* 2006;368:763-70.

63. Grabenhenrich L, Gough H, Reich A, Eckers N, Zepp F, Nitsche O, et al. Early-life determinants of asthma from birth to age 20 years: A German birth cohort study. *J Allergy Clin Immunol* 2014;2014:979-88.

64. Illi S, Von Mutius E, Lau S, Nickel R, Niggemann B, Sommerfeld C, et al. The pattern of atopic sensitization is associated with the development of asthma in childhood. *J Allergy Clin Immunol* 2001;108:709-14.

65. Warm K, Hedman L, Lindberg A, Lotvall J, Lundback B, Ronmark E. Allergic sensitization is age-dependently associated with rhinitis, but less so with asthma. *J Allergy Clin Immunol* 2015;136(6):1559-65.e1-2.

66. Simpson BM, Custovic A, Simpson A, Hallam CL, Walsh D, Marolia H, et al. NAC Manchester Asthma and Allergy Study (NACMAAS): risk factors for asthma and allergic disorders in adults. *Clin Exp Allergy* 2001;31(3):391-99.

67. Marinho S, Simpson A, Marsden P, Smith JA, Custovic A. Quantification of atopy, lung function and airway hypersensitivity in adults. *Clinical and Translational Allergy* 2011;1:16-25.

68. Simpson A, Soderstrom L, Ahlstedt S, Murray C, Woodcock A, Custovic A. IgE antibody quantification and the probability of wheeze in preschool children *J Allergy Clin Immunol* 2005;116:744-9.

69. Carroll WD, Lenney W, Child F, Strange RC, Jones PW, Whyte MK, et al. Asthma severity and atopy: how clear is the relationship? *Arch Dis Child* 2006;91(5):405-9.

70. Sharples J, Gupta A, Fleming L, Bossley CJ, Bracken-King M, Hall P, et al. Long-term effectiveness of a staged assessment for paediatric problematic severe asthma. *Eur Respir J* 2012;40(1):264-78.

71. Lazic N, Roberts G, Custovic A, Belgrave D, Bishop CM, Winn J, et al. Multiple atopy phenotypes and their associations with asthma: similar findings from two birth cohorts *Allergy* 2013;68:764-70.

72. Simpson A, Nevena L, Belgrave DCM, Johnson P, Bishop C, Mills C, et al. Patterns of IgE responses to multiple allergen components and clinical symptoms at 11 years. *J Allergy Clin Immunol* 2015;136:1224-31.

73. ThermoScientific. *ImmunoCAP ISAC- When you need the bigger picture in allergy*. http://www.phadia.com/en/Products/Allergy-testing-products/ImmunoCAP-ISAC/ (accessed 6 Apr 2019).

74. Prosperi MCF, Belgrave D, Buchan I, Simpson A, Custovic A. Challenges in interpreting allergen microarrays in relation to clinical symptoms: A machine learning approach. *Pediatr Allergy Immunol* 2014;25:71-79.

75. Belgrave D, Granell R, Simpson A, Guiver J, Bishop C, Buchan I, et al. Developmental Profiles of Eczema, Wheeze and Rhinitis: Two Population-Based Birth Cohort Studies. *PLoS Med* 2014;11(10).

76. Dharmage SC, Lowe AJ, Matheson MC, Burgess JA, Allen KJ, Abramson MJ. Atopic dermatitis and the atopic march revisited. *Allergy* 2014;69(1):17-27.

77. Kulig M, Klettke U, Wahn V, Forster J, Bauer C-P, Wahn U, et al. Development of seasonal allergic rhinitis during the first 7 years of life. *J Allergy Clin Immunol* 2000;106:832-9.

78. Illi S, Von Mutius E, Lau S, Nickel R, Gruber C, Niggemann B, et al. The natural course of atopic dermatitis from birth to age 7 years and the association with asthma. *J Allergy Clin Immunol* 2004;113:925-31.

79. van-der-Hulst AE, Kilp H, Brand PLP. Risk of developing asthma in young children with atopic eczema: A systematic review. *J Allergy Clin Immunol* 2007;120:565-9.

80. Allen KJ, Dharmage SC. The role of food allergy in the atopic march. *Clin Exp Allergy* 2010;40:1439-41.

81. Muraro A, Werfel T, Hoffmann-Sommergruber K, Roberts G, Beyer K, Bindslev-Jensen C, et al. EAACI Food Allergy and Anaphylaxis Guidelines: diagnosis and management of food allergy. *Allergy* 2014;69(8):1008-25.

82. Nwaru BI, Hickstein L, Panesar S, Muraro A, Werfel T, Cardona V, et al. The epidemiology of food allergy in Europe: a systematic review and meta-analysis. *Allergy* 2014;69:62-75.

83. Roberts G, Lack G. Food allergy and asthma--what is the link? *Paediatric Respiratory Reviews* 2003;4(3):205-12.

84. Sicherer SH. Food allergy. *Lancet* 2002;360:701-10.

85. Soares-Weiser K, Takwoingi Y, Panesar S, Muraro A, Werfel T, Hoffman-Sommergruber K, et al. The diagnosis of food allergy: a systematic review and meta-analysis. *Allergy* 2014;69:76-86.

86. Sporik R, Hill DJ, Hosking CS. Specificity of allergen skin testing in predicting positive open food challenges to milk, egg and peanut in children. *Clin Exp Allergy* 2000;30(11):1541-46.

87. Roberts G, Lack G. Food allergy- getting more out of your skin prick tests. *Clin Exp Allergy* 2000;30:1495-98.

88. Sampson HA, Ho DG. Relationship between food-specific IgE concentrations and the risk of positive challenges in children and adolescents. *J Allergy Clin Immunol* 1997;100:444-51.

89. van Veen WJ, Dikkeschei LD, Roberts G, Brand PLP. Predictive values of specific IgE for clinical peanut allergy in children: relationship with eczema, asthma, and setting (primary or secondary care). *Clinical and Translational Allergy* 2013;3:34.

90. Wang J, Liu AH. Food allergies and asthma. *Current Opinion in Allergy & Clinical Immunology* 2011;11(3):249-54.

91. Kulig M, Bergmann R, Tacke U, Wahn U, Guggenmoos-Holzmann I, the MAS Study Group. Long-lasting sensitisation to food during the first two years precedes allergic airway disease. *Pediatr Allergy Immunol* 1998;9:61-67.

92. Wang J, Visness CM, Sampson HA. Food allergen sensitization in inner-city children with asthma. *J Allergy Clin Immunol* 2005;115(5):1076-80.

93. Schroeder A, Kumar R, Pongracic JA, Sullivan CL, Caruso DM, Costello J, et al. Food allergy is associated with an increased risk of asthma. *Clin Exp Allergy* 2009;39(2):261-70.

94. McGowan EC, Bloomberg GR, Gergen PJ, Visness CM, Jaffee KF, Sandel M, et al. Influence of early-life exposures on food sensitization and food allergy in an inner-city birth cohort. *J Allergy Clin Immunol* 2015;135(1):171-78e4.

95. Saarinen K, Pelkonen AS, Makela M, Savilhahti E. Clinical course and prognosis of cow's milk allergy are dependent on milk-specific IgE status. *J Allergy Clin Immunol* 2005;116:869-75.

96. Malmberg P, Saarinen KM, Pelkonen A, Savilhahti E, Makela MJ. Cow's milk allergy as a predictor of bronchial hyperresponsiveness and airway inflammation at school age. *Clin Exp Allergy* 2010;40:1491-97.

97. Roberts G, Patel N, Levi-Schaffer F, Habibi P, Lack G. Food allergy as a risk factor for life-threatening asthma in childhood: a case-controlled study. *J Allergy Clin Immunol* 2003;112(1):168-74.

98. Custovic A, Johnston SL, Pavord I, Gaga M, Fabbri L, Bel EH, et al. EAACI position statement on asthma exacerbations and severe asthma. *Allergy* 2013;68:1520-31.

99. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ATS/ERS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J* 2014;43:343-73.

100. Wenzel SE. Severe asthma: from characteristics to phenotypes to endotypes. *Clin Exp Allergy* 2012;42:650-58.

101. Fitzpatrick AM, Teague WG, Meyers DA, Peters SP, Li X, Li H, et al. Heterogeneity of severe asthma in childhood: Confirmation by cluster analysis of children in National Institutes of Health/National Heart, Lung, and Blood Institute Severe Asthma Research Program. *J Allergy Clin Immunol* 2011;127:382-9.

102. Chang TS, Lemanske RF, Mauger DT, Fitzpatrick AM, Sorkness CA, Szefler SJ, et al. Childhood asthma clusters and response to therapy in clinical trials. *J Allergy Clin Immunol* 2014;133:363-9.

103. Howrylak JA, Fuhlbrigge AL, Strunk RC, Zeiger RS, Weiss ST, Raby BA. Classification of childhood asthma phenotypes and long-term clinical responses to inhaled anti-inflammatory medications. *J Allergy Clin Immunol* 2014;133:1289-300.

104. Schatz M, Jin-Wen Y, Zeiger RS, Chen W, Dorenbaum A, Chipps BE, et al. Phenotypes determined by cluster analysis in severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2014;133:1549-56.

105. Bourdin A, Mollinari N, Vachier I, Varrin M, Marin G, Gamez A-S, et al. Prognostic value of cluster analysis of severe asthma phenotypes. *J Allergy Clin Immunol* 2014;134:1043-50.

106. Corren J. Exacerbation-prone-asthma-intrinsic to severe disease or a unique phenotype? *Clin Exp Allergy* 2013;44:152-53.

107. McDonald VM, Gibson PG. Exacerbations of severe asthma. *Clin Exp Allergy* 2012;42:670-77.

108. Bai TR, Postma DS, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. *Eur Respir J* 2007;30:452-56.

109. O'Byrne PM, Pedersen S, Tan WC, Busse WW, START Investigators Group. Severe exacerbations and decline in lung function in asthma. *Am J Respir Crit Care Med* 2009;179:19-24.

110. Murray CS, Poletti G, Kebadze T. Study of modifiable risk factors for asthma exacerbations: virus infection and allergen exposure increase the risk of asthma hospital admissions in children. *Thorax* 2006;61:376-82.

111. Forno E, Celedon JC. Predicting Asthma Exacerbations in Children. *Current Opinion in Pulmonary Medicine* 2012;18(1):63-69.

112. Reddel HK, Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW. An official American Thoracic Society/European Respiratory Society Statement: Asthma Control and Exacerbations *Am J Respir Crit Care Med* 2009;180(1):59-99.

113. Sears MR. Can We Predict Exacerbations of Asthma? *Am J Respir Crit Care Med* 2019;199(4):399-400.

114. Kupczyk M, ten Brinke A, Sterk PJ, Bel EH, Papi A, Chanez P, et al. Frequent exacerbators- a distinct phenotype of severe asthma. *Clin Exp Allergy* 2013;44:212-21.

115. SIGN/BTS. *British guideline on the managment of asthma- A national clinical guideline*. www.sign.ac.uk/assets/sign153.pdf (accessed 24 Apr 2019).

116. Miller MK, Lee JH, Miller DP, Wenzel SE, for the TENOR Study Group. Recent asthma exacerbations: A key predictor of future exacerbations *Respiratory Medicine* 2007;101:481-89.

117. Hasselkorn T, Zeiger RS, Chipps BE, Mink DR, Szefler SJ, Simons FER, et al. Recent severe asthma exacerbations predict future exacerbations in children with severe or difficult-to-treat asthma. *J Allergy Clin Immunol* 2009;124:921-7.

118. Bloom CI, Palmer T, Feary J, Quint JK, Cullinan P. Exacerbation patterns in adults with asthma in England: a population-based study. *Am J Respir Crit Care Med* 2019;199(4):446-53.

119. Meltzer EO, Busse WW, Wenzel SE, Belozeroff V, Weng HH, Feng J, et al. Use of the Asthma Control Questionnaire to predict future risk of asthma exacerbation *J Allergy Clin Immunol* 2011;127:167-72.

120. Robroeks CM, van Vliet D, Jobsis Q, Braekers R, Rijkers GT, Wodzig WKWK, et al. Prediction of asthma exacerbations in children: results of one-year prospective study. *Clin Exp Allergy* 2012;42:792-98.

121. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control *Eur Respir J* 1999;14:902-7.

122. Wei H-H, Zhou T, Wang L, Zhang H-P, Fu J-J, Wang L, et al. Current asthma control predicts future risk of asthma exacerbation: a 12-month prospective cohort study. *Chin Med J* 2012;125(17):2986-93.

123. Leung TF, Ko FWS, Wong GWK, Li CY, Yung E, Hui DSC, et al. Predicting Changes in Clinical Status of Young Asthmatics: Clinical Scores or Objective Parameters? *Pediatric Pulmonology* 2009;44:442-49.

124. Fuhlbrigge AL, Weiss ST, Kuntz KM, Paltiel AD. Forced Expiratory Volume in 1 Second Percentage Improves the Classification of Severity Among Children With Asthma *Pediatrics* 2006;118(2):347-55.

125. Wu AC, Tantisira K, Lingling L, Schuemann B, Weiss ST, Fuhlbrigge AL, et al. Predictors of Asthma Symptoms Are Different From Predictors of Severe Exacerbations From Asthma in Children *Chest* 2011;140(1):100-07.

126. Rao DR, Gaffin JM, Baxi SN, Sheehan WJ, Hoffman EB, Phipatanakul W. The Utility of Forced Expiratory Flow between 25% and 75% of Vital Capacity in Predicting Childhood Asthma Morbidity and Severity. *Journal of Asthma* 2012;49(6):586-92.

127. Bloom CI, Nissen F, Douglas IJ, Smeeth L, Cullinan P, Quint JK. Exacerbation risk and characterisation of the UK's asthma population from infants to old age. *Thorax* 2018;73:313-20.

128. Ramsahai JM, Hansbro PM, Wark PAB. Mechanisms and Management of Asthma Exacerbations *Am J Respir Crit Care Med* 2019;199(4):423-32.

129. Mahut B, Trinquart L, Delclaux C. Influence of Age on Risk of Severe Exacerbation and Asthma Control in Childhood. *Journal of Asthma* 2011;48:65-68.

130. Sears MR. Epidemiology of asthma exacerbations *J Allergy Clin Immunol* 2008;122:662-68.

131. Ortega H, Miller DP, Li H. Characterization of Asthma Exacerbations in Primary Care Using Cluster Analysis. *Journal of Asthma* 2012;49:158-69.

132. Just J, Gouvis-Echraghi R, Rouve S, Wanin S, Moreau D, Annesi Maesano I. Two novel, severe asthma phenotypes identified during childhood using a clustering approach. *Eur Respir J* 2012;40:55-60.

133. Arasi S, Porcaro F, Cutrera R, Fiocchi A. Severe Asthma and Allergy: A Pediatric Perspective *Front Pediatr* 2019;7:28.

134. Custovic A, Simpson A, Bardin PG, Le Souef PN. Allergy is an important factor in asthma exacerbation: A Pro/Con Debate. *Respirology* 2010;15:1021-27.

135. Keil T, McBride D, Grimshaw K, Niggemann B, Xepapadaki P, Zannikos K, et al. The multinational birth cohort of EuroPrevall: background, aims and methods. *Allergy* 2010;65(4):482-90.

136. Nickel R, Niggemann B, Gruber C, Kulig M, Wahn U, Lau S. How should a birth cohort study be organised? Experience from the German MAS cohort study. *Paediatric Respiratory Reviews* 2002;3:169-76.

137. McBride D, Keil T, Grabenhenrich L, Dubakiene R, Drasutiene G, Fiocchi A, et al. The EuroPrevall birth cohort study on food allergy: baseline characteristics of 12,000 newborns and their families from nine European countries. *Pediatr Allergy Immunol* 2012;23(3):230-9.

138. McLeod S. *Questionnaire*. www.simplepsychology.org/questionnaires.html (accessed 29 Jan 2019).

139. Asher MI, Keil U, Anderson HR, Beasley R, Crane J, Martinez FD, et al. International study of asthma and allergies in childhood (ISAAC): rationale and methods. *Eur Respir J* 1995;8:483-91.

140. Schoemaker AA, Sprikkelman AB, Grimshaw KE, Roberts G, Grabenhenrich L, Rosenfeld L, et al. Incidence and natural history of challenge-proven cow's milk allergy in European children--EuroPrevall birth cohort. *Allergy* 2015;70(8):963-72.

141. Barros A. Alternatives for logistic regression in cross-sectional studies: an empirical comparison of models that directly estimate the prevalence ratio. *BMC Med Res Methodol* 2003;3:21.

142. Fleming L, Murray C, Bansal AT, Hashimoto S, Bisgaard H, Bush A, et al. The burden of severe asthma in childhood and adolescence: results from the paediatric U-BIOPRED cohorts *Eur Respir J* 2015;46:1322-33.

143. Shaw D, Sousa AR, Fowler S, Fleming L, Roberts G, Corfield J, et al. Clinical and inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur Respir J* 2015;46:1308-21.

144. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the Asthma Control Test: A survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.

145. Liu AH, Zeiger R, Sorkness C, Mahr T, Ostrom N, Burgess S, et al. Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol* 2007;119:817-25.

146. Juniper EF, Guyatt GH, Feeny DH, Ferrie PJ, Griffith LE, Townsend M. Measuring quality of life in children with asthma. *Quality of Life Research* 1996;5(1):35-46.

147. Juniper EF, Guyatt GH, Feeny DH, Ferrie PJ, Griffith LE, Townsend M. Measuring quality of life in the parents of children with asthma. *Quality of Life Research* 1996;5(1):27-34.

148. Juniper EF, Guyatt GH, Ferrie PJ. Measuring quality of life in asthma. *Am Rev Respir Dis* 1993;147:832-38.

149. Cohen J, Mann D, Wisnivesky J, Horne R, Leventhal H, Musumeci-Szabo T, et al. Assessing the validity of self-reported medication adherence among inner-city asthmatic adults: the Medication Adherence Report Scale for Asthma. *Annals of Allergy, Asthma, & Immunology* 2009;103(4):325-31.

150. Miller MR, Hankinson J, Brusasco V, Burgos F. ATS/ERS Task Force: Standardisation of Lung Function Testing. *Eur Respir J* 2005;26:319-38.

151. Lefaudeux D, Dr Meulder B, Loza MJ, Peffer N, Rowe A, Baribaud F, et al. U-BIOPRED clinical adult clusters linked to a subset of sputum omics. *J Allergy Clin Immunol* 2017;139:1797-807.

152. The ISAAC Steering Committee. Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema: ISAAC. *Lancet* 1998;351:1225-32.

153. Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez F. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth cohort study. *Lancet* 2008;372(9643):1058-64.

154. Grimshaw KE, Maskell J, Oliver EM, Morris RC, Foote KD, Mills EN, et al. Introduction of complementary foods and the relationship to food allergy. *Pediatrics* 2013;132(6):e1529-38.

155. Magnusson LL, Olesen AB, Wennborg H, Olsen J. Wheezing, asthma, hayfever and atopic eczema in childhood following exposure to tobacco smoke in fetal life. *Clin Exp Allergy* 2005;35(12):1550-56.

156. Erling V, Jalil F, Hanson LA, Zaman S. The impact og climate on the prevalence of respiratory tract infections in early childhood in Lahore, Pakistan. *Journal of Public Health Medicine* 1999;21(3):331-39.

157. Makinen TM, Juvonen R, Jokelainen J, Harju TH, Peitso A, Bloigu A, et al. Cold temperature and humidity are associated with increased occurence of respiratory tract infections *Respiratory Medicine* 2009;103:456-62.

158. Cheng G, Smith AM, Levin L, Epstein T, Ryan PH, LeMasters GK, et al. Duration of day care attedance during infancy predicts asthma at the age of seven: Cincinnati Childhood Allergy and Air Pollution Study. *Clin Exp Allergy* 2014;44:1274-81.

159. Martin PE, Eckert JK, Koplin JJ, Lowe AJ, Gurrin LC, Dharmage SC, et al. Which infants with eczema are at risk of food allergy? Results from a population-based cohort. *Clin Exp Allergy* 2015;45(1):255-64.

160. Roberts G. Emerging Risk and Protective Factors for Asthma. In: Akdis CA, Agache I (eds.) *Global Atlas of Asthma*. Zurich: European Academy of Allergy and Clinical Immunology; 2013 p45-47.

161. Nissen SP, Kjaer HF, Host A, Nielsen J, Halken S. The natural course of sensitization and allergic diseases from childhood to adulthood. *Pediatric Allergy and Immunology* 2013;24(6):549-55.

162. Michel G, Silverman M, Strippoli M-PF, Zwahlen M, Brooke AM, Grigg J, et al. Parental understanding of wheeze and its impact on asthma prevalence estimates. *Eur Respir J* 2006;28:1124-30.

163. Frew AJ. Atopy and Asthma. In: Akdis CA, Agache I (eds.) *Global Atlas of Asthma*. Zurich: European Academy of Allergy and Clinical Immunology 2013 p84-85.

164. Sears MR, Greene JM, Willan AR, Wiecek EM, Taylor DR, Flannery EM, et al. A Longitudinal, Population-Based, Cohort Study of Childhood Asthma Followed to Adulthood. *N Engl J Med* 2003;349(15):1414-22.

165. Barbarot S, Auziere S, Gadkari A, Girolomoni G, Puig L, Simpson EL, et al. Epidemiology of atopic dermatitis in adults: Results from an international survey. *Allergy* 2018;73:1284-93.

166. Ker J, Hartert TV. The atopic march: what's the evidence? *Annals of Allergy, Asthma, & Immunology* 2009;103(4):282-9.

167. Shaaban R, Zureik M, Neukirch C, Heinrich J, Sunyer J, Wjst M, et al. Rhinitis and onset of asthma: a longitudinal population-based study. *Lancet* 2008;372:1049-57.

168. Del-Giacco SR, Bakirtas A, Bel EH, Custovic A, Diamant Z, Hamelmann E, et al. Allergy in severe asthma. *Allergy* 2017;72:207-20.

169. Frith J, Fleming L, Bossley C, Ullmann N, Bush A. The complexities of defining atopy in severe childhood asthma. *Clin Exp Allergy* 2011;41(7):948-53.

170. Green RM, Custovic A, Sanderson G, Hunter J, Johnston SL, Woodcock A. Synergism between allergens and viruses and risk of hospital admission with asthma: case-control study *BMJ* 2002;324:1-5.

171. Wright RJ, Rodriguez M, Cohen S. Review of psychological stress and asthma: an integrated biopsychosocial approach. *Thorax* 1998;53:1066-74.

172. Bush A, Fleming L. Phenotypes of Refractory/Severe Asthma. *Paediatric Respiratory Reviews* 2011;12:177-81.